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U. S. ARMY MEDICAL RESEARCH UNIT

Institute for Medical Research
Kuala Lumpur, Malaysia

ANNUAL PROGRESS REPORT

UNCLASSIFIED

Reports Control Symbol CSCRD-16

1 October 1964 - 30 September 1965

U. S. ARMY MEDICAL RESEARCH UNIT

Institute for Medical Research
Kuala Lumpur, Malaysia

ANNUAL PROGRESS REPORT*

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PROGRESS REPORT

PROJECT: Grant No. DA-MD-49-193-65-G147

TITLE: DISEASES OF POTENTIAL MILITARY IMPORTANCE IN SOUTHEAST ASIA.

NAME AND ADDRESS OF REPORTING INSTALLATION:

Institute for Medical Research
Pahang Road, Kuala Lumpur, Malaysia

NAME OF DIVISION:

U.S. Army Medical Research Unit (Malaysia).

PERIOD COVERED BY THIS REPORT:

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ABSTRACT

NUMBER AND TITLE OF PROJECT:

DISEASES OF POTENTIAL MILITARY IMPORTANCE IN SOUTHEAST ASIA.

NAME OF REPORTING INSTALLATION: U.S. Army Medical Research Unit (Malaysia)
Institute for Medical Research
Kuala Lumpur, Malaysia.

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PROGRESS REPORT

During the period covered by this report, investigations of melioidosis, leptospirosis, scrub typhus, tick typhus, malaria, and migratory animals were undertaken by this Unit.

Melioidosis: it has been shown that the causative micro-organism, Pseudomonas pseudomallei, is found in all states of West Malaysia, and for the first time the organism was isolated from soil and surface water in East Malaysia (Sabah, formerly North Borneo). Cleared areas and wet rice cultivation areas yielded a higher percentage of isolations than other areas studied. Intensive study of an island in the Malacca Straits indicated a relationship between contamination of surface water with the micro-organism and degree of rainfall. Preliminary studies have indicated growth of the micro-organism in soil and detailed studies of this phenomenon are in progress. Epidemiological study of three human cases were undertaken during the period, and infection of zoo animals and domestic and wild mammals was observed. Serologic study of both humans and animals was undertaken.

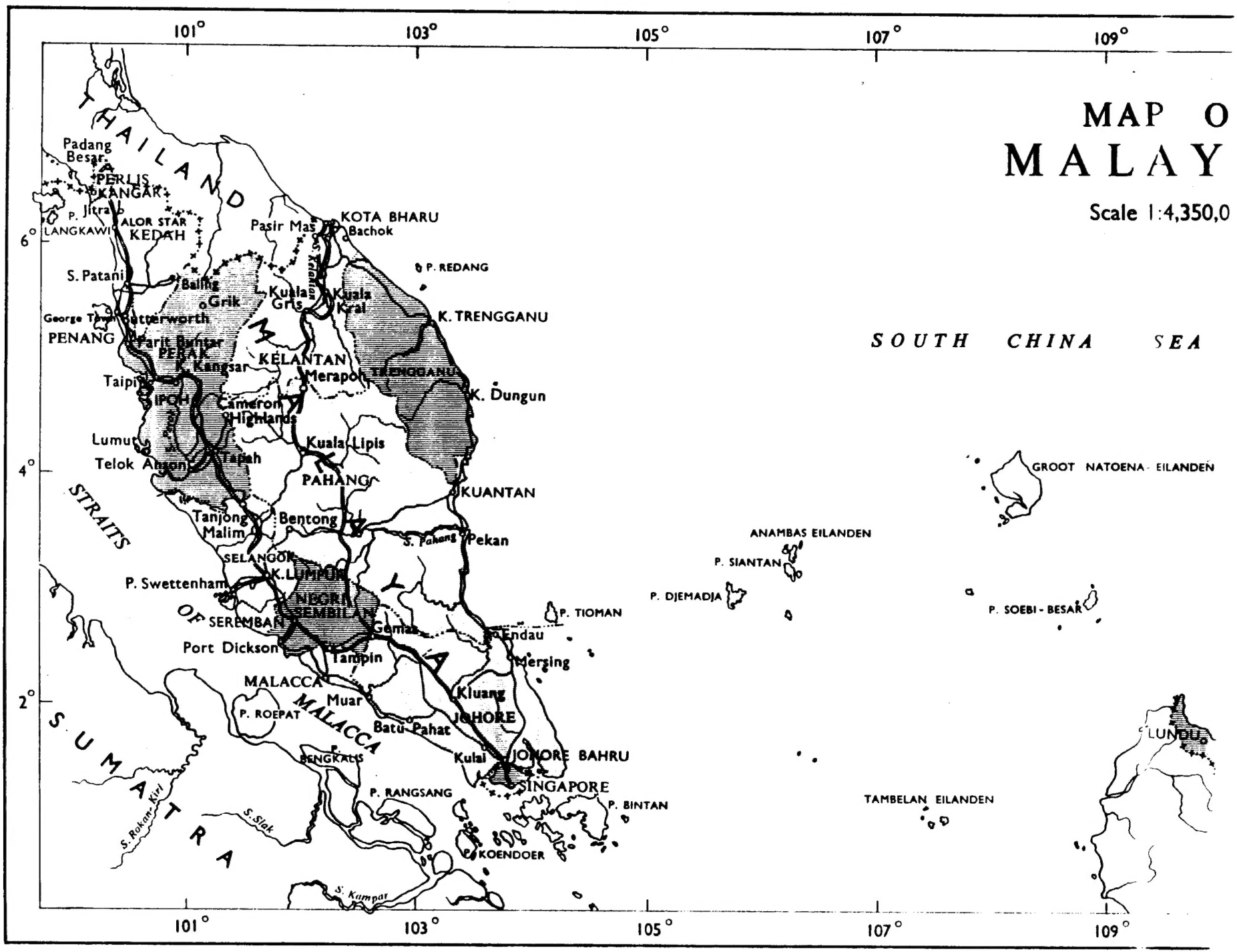
Leptospirosis: studies were begun to determine the influence of age on susceptibility of hamsters to infection with various serotypes of leptospires. Attempts to isolate a large number of leptospires in eastern Sabah failed because of low rainfall at the time of sampling, though two strains were isolated in Tawau Residency.

Scrub Typhus: rickettsiae were isolated from one of three primary forest areas in West Malaysia. The vector L. (L.) deliense was collected in all areas studied. Human disease presumably contracted in three different forest areas was recognized and follow-up studies planned. The immunofluorescence technique was applied to the diagnosis of human scrub typhus, and antibody was demonstrated in all cases of classical disease. Colonies of L. (L.) deliense and L. (L.) akamushi were maintained for the purpose of determining transovarial passage of R. tsutsugamushi. To date these colonies appear to be free of rickettsiae. Studies conducted in Kao Yai National Park, Thailand, with Thai Component, SEATO Laboratory, Bangkok, showed that the distribution of L. (L.) akamushi and L. (L.) deliense on the ground is the same as that observed at Subang, Selangor: L. (L.) akamushi is confined to the grassland and L. (L.) deliense is confined to the forest. Studies of scrub typhus in forest produced two by-products: 1) recognition of at least one of the habitats of forest chiggers - holes, crevices and other irregularities in the forest floor, and 2) six new species of Leptotrombidium chiggers. Studies of the habitat of L. (L.) arenicola, a vector found near sandy beaches, were unsuccessful because study areas near Kuala Lumpur no longer had many chiggers of this species. Other areas will be studied.

Tick Typhus: epidemiologic study of one case of tick typhus was undertaken during the period. Rickettsiae were not isolated though two species of ticks were found in the study area, which are known to harbor spotted fever rickettsiae.

Malaria: studies were designed to investigate the feasibility of inducing in monkeys immunity to malaria by prior inoculation of killed sporozoites. So far the following has been demonstrated in immunized animals: a reduction in the parasitemia, prolongation of the pre-patent period, and modification of the clinical disease.

Migratory animals: more than 20,000 birds were banded in Malaysia. No long distance recoveries have been reported yet. Ectoparasites from the netted birds were studied.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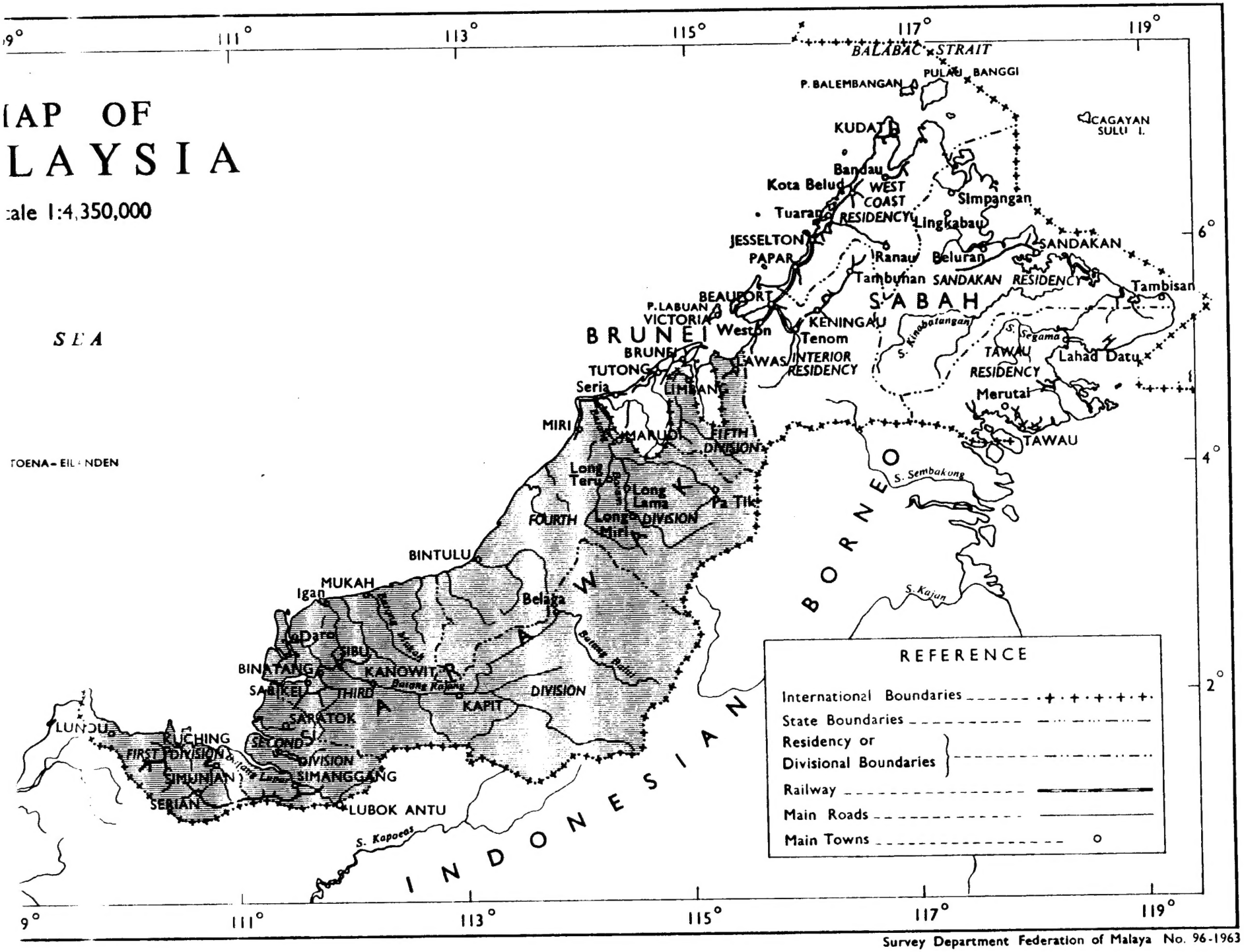


Survey Department Federation of Malaya No. 96-1963

Investigations of Melioidosis

The study of melioidosis as a disease of potential military importance in South East Asia was continued this year by members of the veterinary medicine department. The Division of Veterinary Medicine, WRAIR, WRAMC, Washington DC has given support by confirming the identity of isolated made by the unit, and by supplying the biologicals with which to perform serological tests. Unit melioidosis studies have been coordinated with those done by the SEATO Medical Research Laboratories in Bangkok. Throughout the year Malaysian government veterinary and agricultural officers have been extremely helpful in the collection of study specimens. The management of the Carey Island Estate has been most cooperative in helping the unit in its studies there.

The presence of the causative micro-organism, Pseudomonas pseudomallei in Malaya has been known for more than 40 years. The objectives of the past year's studies have been (1) to determine the distribution of Ps. pseudomallei in nature, throughout Malaysia, both East and West in order to identify the circumstances of high risk of exposure for man; (2) to determine in areas of apparent high endemicity the relative importance of various factors which may influence the distribution and multiplication of Ps. pseudomallei in nature, such as rainfall, soil types, and small mammal infection; (3) to determine by cultural and serological means the extent of involvement of domestic animals, and indigenous and non-indigenous humans with this organism.

A. Distribution of Pseudomonas pseudomallei in soil and water.

A simple procedure for isolating Pseudomonas pseudomallei from soil and water specimens was developed at this unit during the previous report year, and was used this year to process many hundreds of samples taken throughout East and West Malaysia.

1. Methods:

Water from rivers, streams, drainage ditches, padi fields, wells, and rain puddles was collected in small (20 cc) sterile screw cap bottles. Two cc of each sample was inoculated intraperitoneally into each of five hamsters from 25 to 50 days of age. Soil was collected in cups and later flooded with physiologic saline, triturated thoroughly and allowed to settle. Two ccs of the supernatant saline were inoculated intraperitoneally (I.P.) into hamsters. Heart blood of all hamsters dying between day 1 and day 7 post inoculation was cultured on a differential media incorporating crystal violet at a dilution of 1:200,000 in nutrient agar. Experiments done by this unit have shown that from 6 to 10 organisms inoculated I.P. will kill 50% of inoculated hamster, indicating the threshold of sensitivity of our isolation procedure. Water or soil specimens with a contamination level below this threshold will probably not yield Ps. pseudomallei by this technique.

2. Types of Study Areas:

For presentation purposes the sampled areas of Malaya have been divided into six ecotypes, with considerable range of variation within each type: (1) Primary and secondary forests: these include completely undisturbed areas and areas only partially disturbed by timber operations. (2) Plantation areas: these consist almost entirely of rubber or oil palm tree cultivation. Water samples taken in these areas usually came from streams or drainage ditches that flowed between the rows of trees. (3) Cleared areas: these areas were not being utilized for any specific crop, but consisted of pastures, and clearings associated with human habitation, such as a golf course, playing fields, and cleared land awaiting further development. (4) Wet padi rice cultivation areas: these were either small, poorly drained valleys which were formerly swamp jungle, or large plains formed by marine alluvial deposits. Water samples were taken from the complexes of irrigation canals and drainage ditches which serve these areas. The water was usually muddy. (5) Areas of human habitation: these samples were taken from wells, street and backyard puddles, and town drains. (6) Ponds: most ponds were created by tin mining operations.

3. Results: West Malaysia (Malayan peninsula).

Every state of Malaya was visited during the course of the past year except Penang. Soil and water in all states studied were contaminated with Ps. pseudomallei. No attempt was made to compare contamination levels from state to state as the pattern of sampling varied from field trip to field trip. Results by type of area studied are summarized in Table 1.

Table 1

Ps. pseudomallei from soil and water samples in Malaya

Type areas	Number of specimens collected	Number of specimens positive	Percentage positive
Forests	783	20	2.5%
Rubber and oil palm	421	12	2.8%
Cleared and pasture areas	120	48	48.0%
Wet rice padi	392	87	22%
Human habitation	83	6	6%
Tin mining ponds	198	2	2%

From the results in table 1 it is evident that surface water of padi and cleared areas are more contaminated by Ps. pseudomallei than those of areas covered by forest or tree cultivation. Of particular interest are the findings at Terendak Camp a 4 square mile military cantonment in Malacca. Nineteen of 24 water samples collected in the cleared areas of the Camp were positive for Ps. pseudomallei. This area was secondary jungle until cleared for the camp in 1957. In Johore, surface water from a cleared area of an animal husbandry station near Kluang was sampled on two occasions because of outbreaks of melioidosis which had occurred in pigs and goats on the station. During a rainy period 27% of the samples collected were positive in contrast to those collected following two months of relatively little rainfall, of which only 8% were positive. The influence of rainfall on degree of surface water contamination will be discussed in another section of this report. In areas of human habitation most positive samples came from wells. In Kedah 11 wells were sampled and four were positive. These wells were improved to the extent of having cement walls and surface platform to protect against surface water seepage. Additional water samples were taken in special areas, not included in Table 1. In Selangor positive water samples collected from a peat swamp had a pH 3.0.

4. Results: East Malaysia (Sabah, formerly North Borneo)

The Veterinary Department, State of Sabah, has had considerable interest in melioidosis and has independently initiated studies of this disease. Melioidosis had been diagnosed in a herd of pigs in Keningau, Residency of the Interior, Sabah. These pigs had been imported from Australia, and the outbreak occurred following a period of heavy rainfall and flooding of the pastures where they were allowed free range. Also in Keningau some of a flock of sheep imported from Australia developed fatal melioidosis infections. The Veterinary Department, Sabah, at this time engaged the services of two Peace Corps laboratory technicians to develop serological technics for further study of this disease. Melioidosis had never been recognized on the East Coast of Sabah until the fatal infection of an orangutan was diagnosed by a physician in Sandakan in July 1965.

A field trip to Sabah was made by personnel from the Unit's veterinary department to collect water and soil samples from Western, Eastern and Interior Sabah to be processed for isolation of Ps. pseudomallei. Particular attention was to be given to East Coast areas where melioidosis had never been diagnosed. Table 2 shows the results from Sabah, broken down into type areas.

Table 2

Ps. pseudomallei from soil and water samples in Sabah

Type areas	Number of specimens collected	Number of positive	Percentage of positive
Forest	129	1	0.8%
Rubber and oil palm	51	0	
Cleared and pasture areas	90	2	2.2%
Wet rice padi	23	4	17.4%
Human habitation	54	2	3.7%

While Ps. pseudomallei was found in all type areas studied except tree cultivation, the rate of recovery was much lower than in West Malaysia. It should be noted that there had been a long dry period before the sampling was done on the East Coast and in the Interior. Ps. pseudomallei was cultured from a water sample collected in the Tawau area and from a water sample collected in the Lahad Datu area, both in the Tawau Residency of eastern Sabah. At Keningau, in the Interior Residency, Ps. pseudomallei was isolated from surface water on the animal husbandry station where the melioidosis outbreak in pigs had occurred. On the West Coast there was much rain and the padi fields were just being prepared for planting in the flooded river valleys. Ps. pseudomallei was isolated much more frequently.

B. Intensive study of one island for Pseudomonas pseudomallei: Carey Island, Selangor.

Carey Island is an island of approximately 75,000 acres, two thirds of which is cultivated in rubber, oil palm, coconut, and tea, all under one estate management. This island was selected for intensive study because (1) initial surveys had shown the organism Ps. pseudomallei to be present there in high number; (2) it provided a relatively static human population (about 6,000 Tamils and 600 aborigines), (3) essential vital statistics and climate records are kept by the estate management; (4) the estate dispensaries could provide disease prevalance information on the population and study specimens from the labor force; (5) the island is easily accessible to the unit laboratories (1½ hrs by all weather road) and has telephone service. The island has been created recently, in geological time, from delta deposits of the Klang and Langat rivers, which separate the island from the coast of Selangor on the Malacca Straits.

Topographically the island is flat, most areas being below mean high tide level. Effective drainage of the island is accomplished by tidal gates. The soil is mostly clay loam over sand or sand and clay sandwiched. Development of the island for plantation agriculture began in 1910. Study areas were confined to that part of the island cleared and drained more than forty years ago. The studies of Ps. pseudomallei on Carey Island began on 5 January 1965, and have had the following objectives:

1) to determine the extent of contamination of soil and surface water and to evaluate the influence of rainfall on the rate of recovery of the micro-organism.

2) To examine culturally and serologically the small mammal population of the island to determine their role as a natural reservoir.

3) To examine the human and domestic animal population culturally and serologically for evidence of Ps. pseudomallei infection.

Since the beginning of the investigation, 1638 water samples and 195 soil samples have been collected at Carey Island. In a preliminary survey (annual report, September 1964) the organism had been recovered easily from water in ditches draining rubber tree cultivation. Therefore, in the beginning, daily water samples were taken from several ditches draining rubber cultivations. Subsequently collections were extended to include water from other ecotypes on the island. Results are summarized in table 3 by ecotype sampled. Superimposed on the chart is a schematic representation of daily rainfall, precipitation of less than 0.1 inch/24 hrs not recorded.

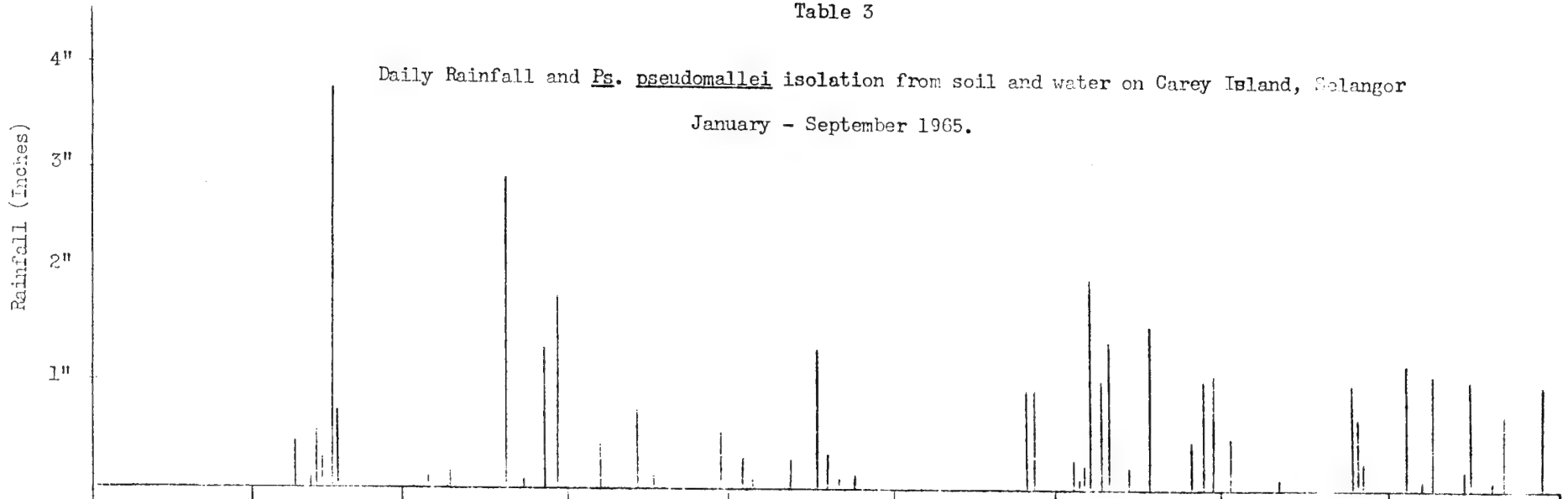
The most striking finding so far is an apparent correlation between the occurrence of rainfall and the degree of surface water contamination. Though water existed almost all the time in the bottom of the drainage ditches, Ps. pseudomallei isolates were made from ditch water only following periods of rain. These periods, as indicated in table 3 have been short in duration and few have occurred in the first 9 months of the project. It is not clear why no organisms were recovered during March and April when some rain fell. At various times the pH of water in the drainage ditches was determined. The pH was found to vary in water taken from the same drain sites but there seemed to have been no correlation between pH and level of contamination by Ps. pseudomallei. The organism was isolated from water having a pH as low as 2.8 and as high as 7.4.

No attempt will be made at this point in the study to compare the level of contamination of water draining the different ecotypes. The organism has been isolated from rubber plot drains, coconut drains, roadside drains next to a primary forest water catchment area, and from a well on the island.

Table 3

Daily Rainfall and Ps. pseudomallei isolation from soil and water on Carey Island, Selangor

January - September 1965.



	January	February	March	April	May	June	July	August	September
Rubber	0/163*	4/165	0/163	1/240	10/282	3/174	9/170	1/50	5/110
Coconut	0/15	0/10	0/70	0/92	1/65	3/47	0/47	0/12	1/23
Forest	0/0	0/0	0/0	0/0	4/26	0/22	2/33	1/12	2/21

* Number of samples positive for Ps. pseudomallei / Number of samples collected, per month

Studies of the small mammal population of the island have failed to detect any involvement with Ps. pseudomallei. Three-hundred and ninety rats have been trapped to date. In the study of the first one hundred rats, urine, feces, heart blood, lung liver, kidney and spleen were cultured with negative results. The remaining trapped rats will only be studied serologically.

Attempts to evaluate nondescript febrile disease in humans on the island for possible melioidosis etiology have been relatively unsuccessful because it was exceedingly difficult to obtain convalescent serum specimens. Paired sera were obtained from only six individuals having FUO and none were found to have antibody. Survey of healthy individuals on the island for melioidosis antibody will be undertaken later in this study.

C. Studies of Pseudomonas pseudomallei in Soil

It has been suggested by some workers that Ps. pseudomallei is a soil saprophyte. Studies were initiated this year to examine the activities of this bacterium in various soils collected at sites which our surveys had shown to be contaminated. At these sites, primarily surface water was processed. Soil was also cultured on occasion but yielded the organism much less frequently.

The procedure for soil experiments is as follows: Soil (5 gm.) to be tested was put in each of a series of screw-capped tubes. Some tubes of soil were then steam sterilized (10 min, 15 lbs). Each tube was seeded with about 100 Ps. pseudomallei organisms. In each experiment, the organisms were added to each tube of soil in 0.5 ml of physiologic saline. Tubes were then capped but not screwed down tightly, and were held undisturbed on the laboratory bench (temperature 70-75°F) until tested for presence of seeded organisms. Some soil samples were partially dried prior to placement in tubes, by exposure to air on the laboratory bench for several days.

Four general soil types were worked with during the past year:

1) Course Sandy Loam

This soil was taken from the banks of a pond just outside Kuala Lumpur, which was formed by tin mining operations in the area. One could describe the soil as "sand" mixed with very little organic matter recently formed. The layer of topsoil was about 2 inches thick. All soil of this type was autoclaved before seeding. There was a definite rise in population of the organism. Two hundred and sixty-three days after seeding, more than 100,000 organisms could be recovered from the soil. Additional tubes of seeded soil remain for future testing.

2) Alluvial Clay - loam soil

Soil was collected from the tops and banks of the rubber plot drainage ditches on Carey Island. Soil of the same type was taken from a patch of forest also on Carey Island. Half the soil tubes were sterilized before seeding. Some of both the sterilized and non-sterilized soil had been air-dried. Growth of Ps. pseudomallei in either sterilized or non-sterilized soil has not been demonstrated in 8 months of observation. (The implications of this finding for studies on Carey Island will be considered in a future report.)

3) Shale derived clay loam soil

This soil was collected at the Central Animal Husbandry Station in Kluang, Johore. It was at this station that the organism was isolated with great ease from pasture drains, streams and ponds.

In this type of soil the organism failed to grow under dry conditions sterilized or unsterilized. In moist soil, the organism multiplied in both sterilized and unsterilized soil. In unsterilized moist soil, Ps. pseudomallei was found in large numbers 36 days following seeding but subsequent tests at later dates failed to isolate the organism. In sterilized moist soil, the organism can still be isolated in large numbers sixty days following seeding. This experiment is still in progress.

4) Laterite soils

This is a free draining soil coming from Terendak Camp in Malacca where the surface water was found to be heavily contaminated with Ps. pseudomallei. Seeded soils, whether sterilized or unsterilized, dry or moist, have yielded Ps. pseudomallei for one month. This experiment is still in progress.

Comment: Results of preliminary studies to determine Ps. pseudomallei activity in soil suggest that the organism multiplies rapidly in certain types of soil, but does not thrive in soil that is dry. The maximum length of time that the organism can survive in a given soil type is not known. The possibility of other factors influencing growth, such as competition from other organisms, is suggested. More definitive experiments will be initiated.

D. Epidemiological studies on Melioidosis

Epidemiological studies of melioidosis have taken two courses, (1) the investigation of the human and animal cases occurring in Malaysia during the past year, and (2) serological survey of humans and animals.

1. Follow-up of human cases of melioidosis.

Case 1. A commonwealth enlisted man, from an army camp in Kluang, Johore (near the Central Animal Husbandry Station) died following a long

illness. Water from various sources on the camp were sampled and the organism was isolated from some of them. Friends of the individual were interviewed concerning personal habits but no activity of his could be specifically incriminated as resulting in his infection. His sole duty was camp storekeeper. The fact that he was an unrecognized diabetic may have been a factor in his susceptibility to this infection. The onset of his illness occurred one month after a period of heavy rainfall at the camp.

Case 2. A cook-sergeant stationed at Terendak Camp in Malacca died after an acute febrile illness. He had had a furuncle incised one month previously. He also was a chronic alcoholic, which may have played a part in the susceptibility of this individual. Water samples collected from various sources within the camp during a rainy spell were about seventy percent positive for Ps. pseudomallei. (Post-mortem tissues and cultures from both cases were forwarded to AFIP and WRAIR for further study).

Eighty-two grounds keepers at Terendak Camp were bled by members of the Unit and four had Hemagglutination (HA) antibody titer for melioidosis of 1:80 or above. Seven had HA titers of 1:40. Studies of human sera from nonendemic melioidosis areas (the USA) using the HA test, suggest that an HA antibody titer of 1:80 or greater may be indicative of past infection with Ps. pseudomallei. An HA antibody titer of 1:40 may be regarded as of borderline significance. Forty buffalo from the same area were serologically tested. Four had HA titers of 1:80 or above, three had titer of 1:40. See table 5.

Case 3. A Commonwealth helicopter pilot developed a respiratory illness on duty in Borneo. Ps. pseudomallei was cultured from sputum by Commonwealth Forces laboratories. The patient recovered from his illness following treatment with chloramphenicol (Roy. Soc. Trop. Med. and Hyg., 59:359, 1965). Serum and a culture from this man was forwarded to USAMRU for further study. Serological findings are included in table 4.

2. Follow-up of Animal Cases

a. Zoo monkeys

Pigtail macaque #1 - Ps. pseudomallei was isolated from a loopful of pleural fluid of a Macaca nemestrina that died at the National Zoo in Kuala Lumpur. There were no abscesses, only congested lungs and liver and an enlarged heart. Melioidosis was not suspected and the carcass was discarded.

Pigtail macaque #2 - About one month following a fight injury, a cagemate of the first animal became weak, and was soon paralyzed in both legs. Four months later, following massage and vitamin therapy, the paralysis disappeared. One month following his recovery from paralysis, an abscess developed over his right hip. Pus aspirated from this large abscess yielded Ps. pseudomallei in pure culture. The animal is being held without treatment, and is observed closely to follow the natural course of the disease.

Water from the river flowing through the Zoo grounds is used for washing down the cages. This water was sampled and Ps. pseudomallei was isolated. Both monkeys had a history of fight wounds and infection could have been due to contaminations of these wounds by the river water.

b. Laboratory monkey (A-21)

A rhesus monkey was splenectomized in conjunction with a malaria experiment done at this unit. At the time of splenectomy a percutaneous liver punch biopsy was performed. Three days later a skin abscess formed at the site of the punch biopsy and Ps. pseudomallei was cultured from this abscess in pure culture. Inspite of treatment with chloramphenicol, the animal died 4 days later. Post mortem examination showed what appeared to be hematogenic nodules throughout the lung tissue. There was a brown caseous abscess at the stump of the spleen which on culture yielded Ps. pseudomallei. The surgical procedures had been performed utilizing standard aseptic technic. The etiology in this case is unknown, but one wonders whether the surgical procedure activated a latent infection of Ps. pseudomallei. Study of the animal's serum showed antibody (Table 4).

3. Serological surveys of humans and animals.

a. Serum specimens from human and animal cases of melioidosis have been studied by three different serologic techniques. The results are summarized in Table 4.

Table 4

Serology of individual animal and human melioidosis cases

Case	No. days pre or post clinical symptom onset	CF	Tube Agglut	HA
Kluang Enlisted Man	12th day post	1:20	-	-
	28th day post	1:20	1:160 >	1:320>
	36th day post	1:20	1:320 >	1:320>
Malacca Enlisted Man	2nd day post	1:20	1:160	1:320
	9th day post	1:20	1:320 >	1:320
Helicopter pilot	convalescent serum 14 days post	-	< 1:10	< 1:80
Monk A-21	100 days pre	1:5	±1:10	-
	3 days pre	1:5	±1:10	-
	4 days post	1:20	±1:10	-
#2 Zoo Pigtail	200 days pre		1:40	
	125 days post	1:160	1:180	1:640>

b. Field trips to various areas of Malaysia provided opportunities to bleed both animals and humans:

1) 155 buffalo were bled, because of their liking of ponds from which Ps. pseudomallei was often isolated. Blood was also received from abattoirs.

2) Goats at the Central Animal Husbandry Station, Kluang, had died of melioidosis, so the 27 surviving goats from the same herd were bled.

3) 27 sheep of a flock were bled at the Animal Husbandry Station of Keningau, Sabah, that had suffered an outbreak of melioidosis.

4) 50 cattle at C.A.H.S. were bled since surface water from the farm was found to be heavily contaminated. Seven additional cattle were bled at Jesselton, Sabah.

5) 13 orangutans at Sandakan, Sabah, were bled following the death of one in a colony, due to melioidosis infection.

6) 82 sera from pigs slaughtered in Perak and Kedah on the west coast of Malaya were tested with negative results.

7) Seven dogs, 9 cats, an elephant, and a panther have also been tested serologically, all with negative results.

8) Human sera have been collected from the Carey Island dispensary patients seen because of fever. Fourteen Animal Caretakers at the Central Animal Husbandry Station at Kluang were bled. One of them had an HA antibody titer of 1:160. This individual was thought to have had pulmonary Tuberculosis 7 months prior to the bleeding on the basis of radiological findings. No acid-fast organisms were cultured from his sputum. Sera has been also collected at the Gombak Aborigine Hospital. Eighty-two grounds keepers at Terendak Camp in Malacca were bled in conjunction with studies done following the death of an enlisted man with melioidosis.

The results of the melioidosis serological survey are shown in Table 5.

Table 5

Summary of Melioidosis Serological Survey

Species	Location	Number of samples	Number with HA titer 1:80 or above	% of total	Number with HA titer 1:40 (1:40 HA Test)	% of total
Buffalo						
	Ipoh Abatt.	30	1	3%		
	Kedah	17	2	12%	1	6%
	Kelantan	17	3	18%		
	Malacca Abatt.	40	4	10%	3	7.5%
	Jesselton Abatt.	51	1	2%	1	2%
Goats						
	Kluang CAHS	27	1	3.7%		
Sheep						
	Keningau	27	3	11%	4	15%
	Kelantan	5	1	20%		
Cattle						
	Kluang C.A.B.S.	50	0	0		
	Jesselton	7	1	14%	1	14%
Orang-hutan						
	Sandakan	13	0		0	
Orang-orang (humans)						
	Kluang Animal Caretakers	14	1	7%	0	0
	Carey Island residents	69	0	0	3	6.5%
	Aborigines at Gombak Med. Ctn.	40	1	2%	0	
	Terendak Camp groundskeepers	82	4	5%	7	8.5%

Investigations of Leptospirosis

During the year a study of leptospirosis in monkeys was concluded and investigation of leptospirosis infection in hamsters was begun. Also surface waters of Eastern Sabah were examined for leptospires for the first time.

1. Influence of age on susceptibility of hamsters to infection with various serotypes of leptospires.

Considerable study has been made during previous years of various regimens for isolation of leptospires from mammals, soil and water by hamster inoculation. In the examination of raw jungle water by intraperitoneal inoculation of hamsters, most isolations (90%) could be achieved, most efficiently, by studying thoroughly only those hamsters dying between the 5th and 14th days after inoculation. Further tests of this regimen at WRAIR, employing serologic as well as cultural methods, confirmed the efficiency of the regimen. Relatively few serotypes of leptospires were isolated from water in East Malaysia (North Borneo, April - May 1963) in marked contrast to the diversity of serotypes isolated in Malaya. Twenty to twenty-five day old hamsters were used in Malaya and 45-55 day old hamsters were used in North Borneo.

During the year, the senior laboratory technician, Mr. M. Mariappan, began experiments to determine the effect of hamster age on the susceptibility to infection and on pattern of clinical response to infection with leptospires from contaminated river water. The effect of hamster age on the serotypes of leptospires isolated is also to be determined. The Gombak river at the aborigines medical center was chosen as the source of water. Samples were collected in bottles attached to 3 sampler stakes placed so that only flood-stage water filled the bottles. Flood water entered the bottles on 37 of 156 days between 28 April and 30 September 65. Two cc. of each water sample were inoculated into four or five hamsters of each of the two age groups. Study for leptospires was made of all hamsters dying between the 6th and 16th post-inoculation days. Results to date are summarized in the following table:

Table 6

Number of sampling days	37
Number of samples collected	101
Number of hamsters inoculated	1082
20-25 day old hamsters: number of water samples containing leptospires	31
45-55 day old hamsters: number of water samples containing leptospires	29
Number of water samples found to contain leptospires in both groups of hamsters	19

No significant difference in rate of recovery of leptospires from the Gombak River was observed in hamsters of the two age groups. All leptospiral strains have been forwarded to WRAIR for serotyping, the second aspect of this study.

2. Induced Leptospirosis in Monkeys: evaluation of various therapeutic regimens.

In the preceding year, studies were initiated to attempt to infect monkeys with leptospires. If monkeys could be infected, with illness ensuing, then various therapeutic regimens could be evaluated in the monkeys. Two types of monkeys were used for these studies: Macaca mulatta (Rhesus) and Macaca nemestrina (Pigtail). Twenty-three monkeys were exposed to eight different leptospiral serogroups by various routes. None developed clinical illness. Full details of the work are contained in last year's report. As part of the study, all animals' urine was studied for leptospires for approximately 60 days post-inoculation. Three animals became shedders of leptospires in the urine. One animal showed leptospiruria on the 24th day post-exposure (infecting strain L. javanica), another on the 24th and 31st days (infecting strain L. australis), and a third on the 31st and 51st days (infecting strain L. hebdomadis). Because the main objective of these experiments, to induce a leptospiral illness in monkeys, was not achieved, the study was discontinued in December 1964, and no further investigation of leptospiruria was conducted.

3. Leptospiral Isolation Attempts in Eastern Sabah, August, 1965.

In August, 1965, in conjunction with studies of melioidosis in Sabah, attempt was made to isolate leptospires from soil and water collected along banks of jungle streams in the Eastern Residencies of Sabah, where leptospiral studies had never been done before. Previous members of this Unit had studied the West Coast and Interior Residencies of Sabah in April and May, 1963, when sixty strains of leptospires were isolated for serotyping. It was hoped to add to this experience.

Most samples collected were inoculated into hamsters on the day of collection. In the Tawau area 72 water and 21 soil samples were collected; in the Lahad Datu area 82 water and 21 soil samples were collected; in the Sandakan area 89 water and 10 soil samples were collected. Only two samples yielded leptospires, both from the Tawau area. One sample was a washing of soil taken from a stream bank in Tajong Forest Reserve, several miles north of Tawau Town. This strain was recovered by culture of the kidneys of an apparently healthy hamster sacrificed 16 days post-inoculation. The source material had been inoculated into the hamster on the day of collection in Tawau. A second strain of leptospire was isolated from water from a stream on Bombalai road near Berut estate office, about 20 miles northwest of Tawau Town. This water sample was sent from Tawau to Kuala Lumpur by air, and was inoculated into hamsters at the Unit four days after field collection. One hamster died 11 days post-inoculation, and leptospires were recovered from liver cultured in Fletcher's medium. In subsequent passage this strain was lost.

The very low recovery rate of leptospires was very disappointing. The weather should have been wet during August 1965 on the east coast of Sabah, but this year rains failed to come in any quantity. It is hoped that another opportunity will arise to study eastern Sabah during the peak rainy season of the year.

Investigations of Scrub Typhus

Studies of scrub typhus are undertaken jointly by the rickettsial and entomology departments. Dr. W.D. Kundin, IMR-ICMRT program, Hooper Foundation of the University of California, is a full-time member of the rickettsial department.

Specific objectives:

1. To examine selected areas of primary forest for scrub typhus rickettsiae.

2. To evaluate immunofluorescence as a technique for epidemiological study of scrub typhus.

3. To determine the transovarial passage rate of scrub typhus rickettsiae in known vector species of mites.

4. Scrub typhus vector habitat studies:

a) to define the habitats of L. (L.) deliense in the presence and absence of L. (L.) akamushi.

b) to define the habitat of L. (L.) arenicola.

5. To examine the normal mouse colony for involvement with Encephalitozoon cuniculi.

A. Forest Scrub Typhus

Usual circumstances for encountering scrub typhus infection are well known. It is believed that human scrub typhus infection may also occur as a result of activity in primary jungle, but the source of the infection is not clearly defined, nor are the circumstances of maximum risk known. As early as 1948, a former staff member of the IMR observed that a known vector of scrub typhus, L. (L.) deliense, was carried in considerable numbers by rats trapped in Ulu Langat Forest Reserve, near Kuala Lumpur. During World War II, teams investigating scrub typhus, particularly in Burma, heard about cases of scrub typhus which they believed must have been contracted in primary jungle. Yet the lead developed in 1948 could not be followed up because the Emergency in Malaya closed much of the jungle to research activities. Now it is considered appropriate to re-open this line of research. Initial studies included work near Bukit Pakoh (Penderas), Central Pahang, and was followed by intensive study of three areas of primary forest, one in Ulu Langat Forest Reserve and two in the Cameron Highlands.

1. Preliminary Studies: Bukit Pakoh (Penderas), Pahang.

The Annual Report (30 September 1964) describes recovery of many strains of R. tsutsugamushi from chiggers and small mammals collected in May 1964, from lallang, scrub, and abandoned vegetable gardens near the aboriginal settlement of Penderas. Further collections were made in the same areas in August, 1964. Collection data are summarized in Table 7. Identification of all of the strains recovered in August as R. tsutsugamushi has been confirmed. Both field trips to Penderas were made primarily to study the aborigines for malaria. Recovery of such a large number of strains of scrub typhus rickettsiae was an incidental by-product. Attempts to capitalize on the discovery of such an intense focus of scrub typhus follow two lines: 1) examination of neighboring forest for scrub typhus, and 2) examination of serum of resident aborigines for scrub typhus antibody. The serologic studies have not yet been undertaken.

Approximately one-half mile north of proven foci of scrub typhus at Penderas lies a ridge of hills, of moderate slope, approximately 900 feet in elevation, covered with secondary forest. One hill area, deep enough into forest so as not to be fringe habitat, was selected for study, 1) to determine whether L. (L.) deliense could be found in it, on the ground, as well as on small mammals, and 2) to attempt to demonstrate rickettsiae in forest at known distance from proven scrub typhus foci. In addition, this forest area provided an excellent site to try out field study methods in preparation for studies of primary forest reached with greater difficulty.

Table 8 outlines collections made during four days in October. All material was returned live to Kuala Lumpur each day for processing. In summary, L. (L.) deliense was obtained from trapped animals and from the ground, but in small numbers. Only twice during 30 man-hours of collecting was a cluster of chiggers encountered, composed of five chiggers in one instance and 12 chiggers in another. The other 231 L. (L.) deliense chiggers were collected singly from the forest floor. Gahrliepia and Schongastia (Walchiella) spp., encountered on mammals, were not recovered on black plates. All inoculated material was found to be free of rickettsiae. Certainly, to be representative, collections in forest must be on a much larger scale then was accomplished at Bukit Pakoh.

Table 7

Summary of Mammals, Chiggers and R. tsutsugamushi strains
Penderas, Pahang, May and August 1964

Date	Mammals		Engorged Chigger Pools		Unengorged Chigger Pools	
	No. trapped	No. Pos. for R.t.	No. Prepared[2]	No. Pos. for R.t.	No. Prepared	No. Pos. for R.t.
5-8 May 1964	36[1]	19	30[3]	21	7[4]	0
18-22 August 1964	58[1]	33	58	38[5]	15[6]	6

1. Almost exclusively R. jalorensis and R. argentiventer, all processed individually.

2. Each pool contained all chiggers found on individual rat.

3. Small (5%) sample from each pool: L. (L.) deliense

4. From area different from trapping areas.

5. Taxonomic sample identification (10-20% of pool):

   36 pools - mixed species, predominantly L. (L.) deliense
   1 pool (3/20) - 1 Eutrombicula wichmanni; 2 Schongastia vieta
   1 pool (4/53) - 2 Eutrombicula wichmanni; 2 Schongastia vista

6. Nine negative pools from same negative area studied in May.
   Six positive pools from trapping areas.

Table 8

Summary of Mammals and Chiggers
Forest, Bukit Pakoh, Pahang
6 - 9 October, 1964.

Mammals[1]	L. insicgnus	- 4
	R. sabanus	- 3
No. chigger pools from mammals		- 7 (1/animal)
	No. pools without L. (L.) deliense	- 1 (Gahrliepia sp.)
No. chiggers (all L. (L.) deliense) ground collection		- 248
	No. fed singly on mice	- 40
	No. pools inoculated from remainder	- 2

1. Total trap nights = 706

2. Cameron Highlands: Mt. Brinchang and Upper Sungei* Telom Valley.

In this region, large areas of primary forest at higher altitudes (over 5000 feet) are easily accessible by all-weather roads and are within fifty miles of frequent daily rail service. Two areas were selected for initial study: 1) Mount Brinchang, site of many previous USAMRU field collections, and 2) Sungei Telom, Pahang / Sungei Plau'ur, Kelantan, water diversion scheme area. The latter area, beyond Blue Valley Tea Estate, has been entered for the first time during the past 18 months for construction of a water diversion tunnel across the Kelantan-Pahang watershed divide. A road, open only to hydro-electric personnel, has been built to the divide. One hundred yards on either side of the two mile access road and immediately across the Sungei Plau'ur from the diversion tunnel intake lies primary forest. The field team of MAPS (Lord Medway, University of Malaya) accompanied the USAMRU group to collect both resident and migratory birds. Resident ground birds as well as migratory species, and their larval mites, were to be studied for rickettsiae.

It is possible that in the forest larval mites other than L. (L.) deliense are infected with R. tsutsugamushi. Chigger pools collected on this field trip were prepared with this possibility in mind. Chigger from each animal were kept separate, and sorted into pools homogeneous as to species to the limit possible by x40 magnification under a dissecting microscope. A pre-determined number of chigger (>100 : 10%; 50 - 100: 20%; 25 - 49: ten specimens; 10 - 24: 40%; <10:50%) were removed from each pool and mounted in the field for definitive identification. Since R. tsutsugamushi has been recovered in this laboratory from a single engorged L. (L.) deliense triturated and inoculated i.p. into a mouse, no identification sample short of 100% of a pool of chiggers is large enough to rule out the contamination of a pool with known vectors of scrub typhus. Nevertheless, by the sampling scheme described, it is possible to have reasonable confidence in species composition of chigger pools, and, in the event of isolation of R. tsutsugamushi, to choose which chigger species should be studied more intensively in the laboratory for vector capability.

Table 9 summarizes mammal collections. Table 10 summarizes ectoparasite collections. In contrast to the study at Bukit Pakoh, Pahang, all chigger pools were inoculated into mice in the field, as were tissues of all rats which did not survive thorough check for ectoparasites. Live rats and inoculated mice were shipped at night to Kuala Lumpur at the mid-point (by train) and at the end of the study period (by road). All animals withstood shipment well. From material collected in Cameron Highlands many larval mites previously recorded from high elevations (over 5,500 ft) were identified. A single L. (L.) scutellaris chigger was recovered from a R. edwardsi trapped at the head waters of the Sungei

* Sungei is the Malay word for river.

Telom on the Ulu Kelantan border. Traub and Nadchatram had previously recovered L. (L.) scutellaris from rats trapped on Mount Brinchang. Chiggers were found on all but one rat (R. jalorensis). Review of mounted specimens reveals very few L. (L.) deliense in one instance recovered from Pitta sordida cuculotta, collected at a much higher altitude (6660 feet) than has usually been recorded, and in another instance from a R. alticola also collected on Mt. Brinchang. Predominant chigger species were: Gahrliepia (Walchia) disparunguis pingue and one new Leptotrombidium sp., (see Scrub Typhus section B).

No rickettsiae were found in this material, although two vector species of Leptotrombidium chiggers were found.

Table 9

Summary of Mammals, Birds and Chiggers, Cameron Highlands
28 November - 3 December 1964.

Mammal and bird Species.	Number Trapped	Number netted	Chigger Pools from Mammals and Birds								Total Identified Chigger Pools
			Unmixed sample			Predom. species in mixed sample				Incomplete ident.	
			G. (W) disp. pingue.[3]	L. (?) n. sp. "A"[3]	Other species	G. (W) disp. pingue[3]	L. keuk[3]	L. n. sp. "A"[3]	Other species.		
Mt. Brinchang[1]											
R. fulvescens	26		19	3	0	4[5]	0	0	0	1	27
R. alticola	22		16	1	1[4a]	4	0	0	0	1	23
R. edwardsi	5		1	5	3[4b]	0	0	2	3[7]	0	14
R. bowersi	1										0
R. jalorensis	1										0
Turdus obscurus		3									0
Zoothera sibericus		3									0
Pitta sordida cucullata		4	0	0	2[4c]	0	0	0	2[4c,6]		4
S. Telom/S. Plau'ur[2]											
R. alticola	6		6	0	0	0	0	0	0	0	6
R. edwardsi	1		0	0	0	0	3	1	0	0	4
R. bowersi	1		0	0	0	0	1	0	0	0	1
Totals	63	10									79

(continued)

Table 9 (continued)

1. Total Trap nights : 1,328

2. Total Trap nights : 522

3. Species abbreviations : Gahrliepia (Walchia) disparunguis pingue;

L. (L.) keukenschrijveri;

Leptrotrombidium (?) n. sp. "A".

4a. Walchiella impar; 4b. Trombiculindus hastata (2), Walchiella impar (1); 4c. Toritrombicula sp.

5. Sample of one pool contained one L. (L.) deliense

6. Sample of one pool contained three L. (L.) deliense

7. Leptotrombidium (?) n. sp. "G" (2), Gahrliepia (Gahrliepia) cetrata (1)

8. One pool contained small number of L. (L.) langati; a second pool contained L. (L.) langati and one L. (L.) scutellaris.

3. Second Examination: Upper Telom Valley, Cameron Highlands.

During the period 21-27 February 1965, collections at Upper Sungei Telom were again made. Eighteen rats, one squirrel and one bird, as well as 37 chigger pools prepared from trapped animals, and 8 unengorged chigger pools prepared from specimens collected on black plates were examined for rickettsiae. Table 10 summarizes this material. All chigger pools were inoculated into mice on the day of collection. Tissues of small mammals not surviving examination for ectoparasites were immediately harvested and inoculated into mice in the field. The remaining animals were returned live to Kuala Lumpur for study.

No rickettsiae were isolated from this material.

4. Ulu Langat Forest Reserve, Selangor.

This area constitutes one of the few remaining forests immediately adjacent to Kuala Lumpur which has not been selectively logged at some time during the last forty years. Collections were made in February and March, 1965, by aboriginal trappers employed by the Department of Zoology, IMR. Their courtesy in providing us with this material is greatly appreciated. The area of forest trapped lies between 1000 and 2000 feet elevation, and is roughly classified as Hill Dipterocarp Forest. One hundred and twenty-seven small mammals and 169 chigger pools (150 identified, 19 not studied), prepared from larval mites recovered from these mammals, have been examined for R. tsutsugamushi. Details of this material are summarized in Table 12.

Tissues of 7 small mammals and 2 of the chigger pools were found to contain scrub typhus rickettsiae. Three different recent species were found to contain this agent: 4 (of 30) Rattus edwardsi, 2 (of 15) Rattus mulleri, and 1 (of 20) Rattus sabanus. Both pools of chiggers contained L. (L.) deliense along with other chiggers.

Table 10

Collections made Cameron Highlands
21 Feb - 27 Feb 1965

		Composition of Chigger Pools Inoculated for R. tsutsugamushi Isolations													
		Unmixed samples species									Mixed samples(2) predominant species				
	No. trapped (1)	G. (W.) disp. pingue	Sp. A near L.(L.) intermedia	L. (L.) keukenschrijveri	G. (G.) cetrata	W. oudemansi	Traub. domrowi	L. (L.) langati	G. (W.) impar	Totals	G. (W.) disp. pingue	W. lacunosa	L. (L.) keukenschrijveri	G. (G.) cetrata	G. (W.) impar
S. Telom & S. Plauur															
R. alticola	16	16					4			21					1
R. bowersi	1									1			1		
R. edwardsi	1		1		1	1		2		13	1	2	3	2	
Dremomys rufigenis	1			3				1	1	14			6		1
Total	19	16	1	3	1	1	4	3	1	49	1	2	10	2	2

(1) Tissues of all mammals except R. edwardsi were inoculated into mice.

(2) See Table 11 for list of chigger species.

Table 11

List of chigger species not included in Table 10

Sch. (W.) lacunosa

G. (W.) rustica

G. (G.) neterella

G. (G.) insigne

L. (L.) deliense

L. (L.) scutellaris

L. (L.) indica

L. (Tromb.) near hastata

G. (W.) alpestris

G. (W) ventralis

Traub. owenevansi

Table 12

Summary of Collection
made at
Ulu Langat Forest Reserve, Feb - Mar 1965

MAMMAL SPECIES COLLECTED	Total No. trapped[1]	No. mammals with chigger pools not yet identified	General identity of chigger pools prepared for isolation of R. tsutsugamushi								Total identified chigger pools
			Identification sample unmixed[5]				Predominant species in mixed identification[5] sample				
			L. (L.) deliense	Other Leptotrom.	Gahrliepia Sp.	Other	L. (L.) deliense	Other Leptotrom.	Gahrliepia Sp.	Other	
R. rajah	2	1			1						1
R. sabanus	20	1	2	9	20	5	2	7	8	15	68
R. mulleri	15	7	2			2	3	2		6	15
R. edwardsi	30	21	1	4	3	2	1	3	4	6	24
R. cremoriventer	4					1				3	4
Tupaia glis	11	1	2	1	1	4	3	3	4	7	25
Callosciurus nigrovittatus	4					2				1	3
Callosciurus tenuis	1									1	1
Callosciurus caniceps	12[2]	1									0
Callosciurus notatus	15	5				7					7
Chiropodomys gliroides	12[3]										0
Chiropodomys laticaudatus	2	1									0
Hylomys suilus	1						2				2
Totals	129[1]	-[4]	7	14	25	23	11	15	16	39	150

(1) Tissues of all mammals, except Hylomys suilus and one C. nigrovittatus, were inoculated into mice.
(2) Chiggers, not yet examined for identity, found on only one individual.
(3) Two Ascoshongastia (L) indica found on one specimen; others had no chiggers.
(4) Nineteen chigger pools prepared for inoculation from chiggers collected from these animals.
(5) See Table 13 for list of chigger species.

Table 13

List of Chigger species not included in Table 12

L. (L.) keukenschrijveri
L. (L.) sp. "E" (near nakatae)
L. (L.) langati
L. (L.) deliense
L. (Trombiculindus) near hastata
Schoutedinichia bisetosa
Microtrombicula spicea
Gahrliepia (Schongastiella) birella
G. (S.) argalea
G. (G.) cetrata
G. (G.) rutila
G. (G.) fletcheri
G. (G.) neterella
G. (G.) tesselata
G. (G.) ornata
G. (W.) disparunguis pingue
G. (W.) rustica
G. (W.) turmalis
G. (W.) simulata
G. (W.) lewthwaitei
Ascoschongastia (Laurentella) indica
A. (L.) ctenacarus
A. (L.) roluis
A. (L.) canus
A. (L.) audyi
A. (L.) globosa
A. (L.) krishnani
A. (L.) calcar
Schongastia (Walchiella) lacunosa
S. (W.) oudemansi
S. (W.) impar
S. (Helenicula) mutabilis

5. Comment on the forest scrub typhus studies.

To date scrub typhus rickettsiae have been found at only one of the study sites, although small numbers of L. (L.) deliense have been recovered at each site. We think this is an entirely satisfactory result, under the circumstances. When this study was begun, this Unit did not know of any cases of scrub typhus contracted in forests of Malaya, about which accurate information could be gathered. Therefore study sites were selected which satisfied the ecological criterion of primary, undisturbed forest vegetation. Ulu Langat and the Cameron Highlands specifically were selected because they are representative of Malayan primary forests at elevations of two and six thousand feet respectively. Lowland primary forest along the west coast of Malaya has been destroyed, and studies of this type of forest in the eastern state of Pahang were deferred for logistical reasons.

During the course of this year, three instances of human infection with scrub typhus rickettsiae in Malayan forests came to light. Future work will concentrate on these forests. If it is found that the forest areas where the disease may have been contracted contain zones of disturbed vegetation, then these zones will also be studied. Malayan forests contain many pockets of disturbance, some man made, some of natural origin, and these may contain the foci of scrub typhus which superficially might appear to be in primary forest.

Each of the instances of human infection in forest has been checked serologically by this Unit. Briefly these human infections are as follows: 1) disease occurring in a British lady employed as a Red Cross worker at the British Military Convalescent Hospital in the Cameron Highlands. We believe she contracted her disease while on an overnight visit to an aborigine village on the Telom River, about three miles downstream from Kuala Terla. 2) At least 11 scrub typhus cases were contracted by men of 2 Battalion, Malaysia Rangers, while on border patrol duty in north-east Kedah. This area is primary forest, but has been patrolled by security forces for a number of years, so that many pockets of disturbed vegetation exist in the forest. 3) A RAAF helicopter pilot contracted scrub typhus (and malaria) on a jungle survival exercise in remote western Kelantan state. The exercise involved passage down a jungle river, with frequent stops on the river bank. Again, the general area is primary forest, but aborigines have inhabited the river valley for a long time, creating many pockets of disturbed vegetation.

It is hoped to conduct the follow-up epidemiologic studies of each of these scrub typhus leads during the next six months.

B. By-products of Forest Scrub Typhus Studies

1. Identification of a previously unrecognized forest chigger habitats.

During the second field trip to the Sungei Telom area of the Cameron Highlands in February, 1965, it was first observed that considerable

numbers of chiggers appeared on black plates placed in ground holes in the forest. Considerable effort has been made to extend this observation in order 1) to increase our knowledge of the natural habitats of chiggers heretofore found only on forest animals, and 2) to increase the number of forest chiggers available for scrub typhus studies. A field trip to the Cameron Highlands was made in late June for the sole purpose of examining for chiggers many forest holes at elevations ranging from 1500 to 6500 feet.

To date, 35 different species of chiggers have been recovered from holes, depressions, and crevices in the ground in forest. Most of these have not previously been found in the unengorged state, and several have not previously been described (see Table 14). Most of the chiggers found in holes have also been recovered from rats trapped in the forest. Therefore it seems reasonable to suspect that at least some of the chigger-containing holes in the forest are rat holes. A manuscript describing these preliminary observations has been submitted for publication.

It is important to point out that many holes did not appear to contain chiggers when examined with black plates. Studies to date suggest that the natural habitat of Gahrliepian chiggers may be mainly, if not exclusively, confined to the cool, damp, shaded environment of animal holes. It also appears possible that altitude, reflecting perhaps temperature and moisture variations, influences the species distribution of chiggers found in ground holes in the forest.

Studies based on this observation of a previously unrecognized chigger habitat are proceeding along two lines: 1) study of forest chiggers collected from holes for involvement with R. tsutsugamushi; 2) serial observations of a number of specific holes in several forests near Kuala Lumpur to determine a) species of chiggers present, b) numbers of chiggers present at various times, c) occupants of holes, d) climatic and other factors influencing chigger populations in holes.

Table 14

35 species of Trombiculid larval mites recovered
on black plates from ground holes

Chigger Species			Forest Areas				Padi fields
			West coast	Highlands < 4000 ft	Highlands > 5000 ft	Central & East coast	East coast
Gahrliepia	Gahrliepia	cetrata	2				
"	"	darita			5		
"	"	fletcheri	121	66		103	7
"	"	insigne	1	7		25	
"	"	marshi	1			30	298
"	"	ornata				4	3
"	"	neterella			8		
"	"	picta	1			3	
"	"	rutila	23			11	
"	"	sp. (emicata)		42	224		
Gahrliepia	Walchia	brennani				5	
"	"	cuspidata	11				
"	"	disparunguis disparunguis					11
"	"	disparunguis pingue					7
"	"	lewthwaitei				1	
"	"	rustica	123	264	1	334	11
"	"	turmalis	8	7	3		
	Schoengastiella	argalea	91	10		412	
Leptotrombidium	Leptotrombidium	deliense	6	20	2	7	
"	"	muridia		18			
"	"	sp. (gentryi)				248	
"	"	sp. (kundini)				14	
"	"	sp. (rapmundi)				19	
	Trombiculindus	hastata	13				
	"	sp.				1	
	Eutrombicula	wichmanni					16
	Eltonella	eltoni				1	
	Walchiella	impar		1		5	
	"	oudemansi			6		3
	"	sp.				3	
	Schoengastia	vieta					45
	Neoschoengastia	sp. (heynemani)	12				21
	Schoutedenechia	bisetosa	16			7	
	Eltonella	eltoni				1	
	Fonsecia	n. sp.					3

2. Recovery of L. (L.) scutellaris and L. (L.) keukenschrijveri on black plates.

Some black plating on the ground surface in forest has also been undertaken. A few L. (L.) deliense were recovered in the Cameron Highlands, a finding consistent with observations made by previous investigators of this Unit. In the valley of the Upper Sungei Telom, in February, 1965, a banana thicket was examined with black plates which was located next to a clearing made about one year previously for a construction workers' camp. From the ground surface of this thicket, considerable numbers of L. (L.) keukenschrijveri were recovered. Three specimens of L. (L.) scutellaris were also recovered, two from the banana thicket and one from the ground in the forest about thirty yards from the edge of the banana thicket. It is hoped that further, larger collections of unengorged specimens of both chiggers can be made so that an adequate examination of both species can be made for R. tsutsugamushi.

3. New species of Trombiculid larval mites from the forest.

Six new species of Leptotrombidium chiggers have been described from material collected from forest rats and from ground holes. They are: L. (L.) brinchangense from a R. fulvescens, R. edwardsi and R. alticola, L. (L.) malayanum from a R. alticola, L. (L.) insolitum from a R. edwardsi and R. alticola, (all trapped in the Cameron Highlands); L. (L.) rapmundi from a R. edwardsi and on black plates from forest ground holes in Ulu Langat Forest Reserve; L. (L.) gentryi from forest ground holes in Kelantan; and L. (L.) kundini from forest ground holes in Pahang, near Kuantan. A manuscript describing these new species has been submitted for publication.

Also in the course of forest scrub typhus work, chiggers of the genus Toritrombicula were recovered from a Green Breasted Pitta (Pitta sordida cucullata) taken in early December 1964 at the top of Mount Brinchang (6,666 feet). Preliminary studies suggest that these chiggers may represent a new species. Detailed description of type specimens is being prepared.

C. Epidemiologic study of scrub typhus by immunofluorescence.

1. Background: Scrub typhus is not now a public health problem in Malaya. Historically, rubber and oil palm estate workers have been most frequently affected by the disease. But treatment of most febrile patients since World War II with broad spectrum antibiotics at dispensaries, when the blood smear for malarial parasites is negative, has almost eliminated scrub typhus from civilian hospitals. Sporadic cases are still reported, usually severe illnesses with classical signs and symptoms. The sero-epidemiologic study of this disease has never been undertaken in Malaya because a valid serologic procedure has not been available. The classic OXK Weil-Felix test is only applicable to the diagnosis of recent infection. As shown by previous workers of this Unit, OXK agglutinins are

absent in as many as 50% of cases, and, when present, disappear in a few months. Recently, an immunofluorescence test was devised by a former member of this Unit, Dr. Bennett Elisberg, and his associates at WRAIR. Preliminary studies with this technique suggest that specific scrub typhus antibody is detectable for as long as 12 years after infection. Through the kind cooperation of the Department of Rickettsial Diseases, WRAIR, the immunofluorescence technique has been established at this Unit, permitting study of a number of questions which heretofore could not be approached. Work during the past year with this technique has sought answers to three questions: 1) will the immunofluorescence technique detect scrub typhus antibody in all patients with typical disease? 2) what is the incidence of scrub typhus infection in populations living in close proximity to proven scrub typhus foci? 3) Can infection with scrub typhus rickettsiae be asymptomatic?

2. Immunofluorescence procedure.

Three rickettsial strains are presently used in this procedure. More than one strain is used because immunologic heterogeneity among strains of scrub typhus rickettsiae was demonstrated by early workers of this Unit. The strains are Karp (from a patient infected in Dobadura, New Guinea in 1943), Gilliam (from a patient infected in Burma in 1944), and Kato (a strain from Japan). Since both Karp and Gilliam strains were shown, in initial studies at WRAIR, to be capable of detecting antibody in individuals infected in Malaya, it is reasonable to start with these strains in epidemiologic work in Malaya. The procedure itself is performed as outlined in Proc. Soc. Exp. Biol. & Med., 112:568-573, 1963.

3. Routine diagnosis of human infection.

Serum was studied during the year from 29 patients with infection diagnosed by the clinicians as probable scrub typhus. In every case antibody was detected in the immunofluorescence test. An increase in antibody was not demonstrated in every case because in some instances the acute phase serum was taken quite late, and in other instances only a convalescant serum was sent to us. In all cases antibody was detected with either Karp or Gilliam strains of rickettsiae. No individual's serum contained only antibody to Kato strain, though some individuals with antibody to one or both of the other strains also had antibody to Kato strain. Serial blood specimens are being taken from two of the patients in an effort to follow, with the passage of time, changes in the pattern of antibody response to the three test strains of rickettsiae.

4. Serologic survey of population living near scrub typhus focus: studies at Kota Lama Kanan, Perak.

In March, 1964, a febrile illness in a 21 year old female padi worker living in Kota Lama Kanan district was diagnosed as scrub typhus, on the basis of typical clinical disease and OXK agglutinin response. In May, 1964, this Unit collected small mammals and their ectoparasites in the most likely exposure area: fringe of a padi field. From one of five

rats trapped, R. tsutsugamushi was isolated; nine pools of L. (L.) deliense chiggers were negative for rickettsiae. In July, 1964, serum specimens were obtained from more than 300 residents of the patient's kampong and neighboring kampongs for an antibody survey.

In the immunofluorescence procedure, 50% of 82 sera from residents of Kg. Pauh were reactive at a dilution of at least 1:20 with one or more of the three reference strains. Twenty-five per cent of 88 sera from Kg. Kerdang had antibody. Only 5% of 128 sera from school children had antibody.

Inspite of the relatively high rate of antibody found in this population, the serologic procedure could still have missed antibody in additional individuals if the strains of rickettsiae infecting these individuals were unrelated to the reference strains used in the test. Accordingly, additional small mammal and chigger collections were made in the immediate vicinity of the sentinel case's padi field. Collections are summarized in Table 15. Sixteen rats and 7 L. (L.) deliense chigger pools yielded scrub typhus rickettsiae. Antibody produced in mice to all 23 strains was detected in the immunofluorescence procedure by one or more of the reference strains. Attempts are still being made to evaluate antibody to these strains produced in guinea pigs, feeling that the results in mice are not necessarily applicable to the question of cross-reactivity in man.

Table 15

Summary of Mammals and Chiggers
Kg. Kota Lama Kanan, Perak
16 - 18 November 1964.

Mammal Species[1]	Number Trapped[2]	Chiggers from Mammals			Number chigger pools inoculated
		Number mammals with L. deliense only[3]	Number mammals with L. deliense and others[3]	Number mammals with no L. deliense	
R. jalorensis	28	15	13	0	34
R. rajah	3	0	0	2	2
R. argentiventer	2	1	1	0	3
R. diardi	1	1	0	0	1
Tupaia glis	4	0	4	0	7
	38				47

1. Number of Trap-nights: 419
2. All mammals, except 2 Tupaia glis which died, processed individually for rickettsiae.
3. On basis of taxonomic sample identification.

5. Search for evidence of inapparent scrub typhus infection.

In December 1964, a 10 year old British school child contracted scrub typhus near Tampin, Negri Sembilan. It was presumed that he contracted his disease near his home, and therefore it was possible that other school children might have been exposed to the disease. No other scrub typhus infection was manifest in the approximately 150 other British school children living near Tampin. Accordingly, a study was made of these children looking for evidence of inapparent scrub typhus infection.

The housing and recreational areas frequented by the children were surveyed for scrub typhus vectors. Tapioca-baited traps were set one night for small mammals and all likely areas were sampled by black-plating technique for chiggers on the ground. One large area had been sprayed with miticide shortly after the affected child became ill. No vectors were found here by black-plating, and two rats, both R. jalorensis, trapped in the area were free of chiggers. In one lalang field next to a housing area, a focus of L. (L.) akamushi was found, 26 specimens being recovered in 15 minutes, by four men exposing 10 plates each for periods of approximately 3 minutes each. Other grassy areas near housing did not contain vector chiggers, on the basis of similar sampling. Near the school recreational area, one lalang covered slope and the wooded sides of a gully were examined for vector chiggers. One L. (L.) deliense was recovered from a wooded zone of the gully, whereas the lalang did not yield chiggers. Thus two vector species of chiggers were present but in very small numbers.

Capillary blood samples were obtained from 133 school children, including the sentinel case. The distribution of specimens by sex and age is as follows:

Age	5	6	7	8	9	10	11	Totals
Male	7	17	14	11	8	5	4	66
Female	15	12	13	9	6	6	6	67

Serum of one child, C.Q., reacted in the scrub typhus immunofluorescence test, and the serum of a second child, S.B., had partial reaction with one of three test strains of rickettsiae:

	Child	Age	Scrub strain:	Karp	Gilliam	Kato
	C.Q.	9		+1:10	neg 1:10	neg 1:10
	S.B.	5		-1:10	neg 1:10	neg 1:10
(sentinel case)	K.S.	9	(onset 23 Nov 64)			
			30 Nov 64	1:10	neg 1:10	neg 1:10
			14 Dec 64	>1:640	>1:160	1:80
			1 April 65	>1:640	1:160	1:40

The child C.Q. had had a self-limiting three day febrile illness one month before the survey blood specimens were obtained. At that time the child did not have an eschar or any other localizing sign or symptom. It

is possible but, we think, unlikely that she had scrub typhus infection at this time.

During the two brief periods of our investigation, we observed many children, some as young as 2-3 years of age, walking in the scrub vegetation. They tended, however, to keep to areas of bare ground or places covered with very short grass. In short, they tended to avoid the few areas where vectors would be expected to occur. Thus it is likely that only very rarely were children exposed to vector chiggers. The serologic findings of antibody in only one child tends to confirm this. Unfortunately, this child left Tampin for home shortly after the survey specimens were taken, and she will not return to Malaya.

Other situations suitable for study of this question will be sought in the future.

D. Sandy beach, shoreline scrub typhus habitat.

In March, 1965, efforts were begun to locate the natural habitat(s) of L. (L.) arenicola. A number of years ago, R. tsutsugamushi was isolated from chigger pools believed to be exclusively composed of this chigger. It is suspected that at least one outbreak of scrub typhus has been caused by this species of chigger. This chigger has been found up to now only on rats, and only on those trapped within about 50 yards of a sandy shoreline. So far, the only shore areas shown to possess this chigger have been 1) the coast of S.E. Johore, 2) Pangkor Island, and 3) Morib Beach, Selangor, 4) Pulau Langkawi. It was decided to begin work again at Morib.

Initially, trapping of small mammals was undertaken at two beach fringe sites, one of which (Sultan's Beach House area) had been studied briefly in September, 1963, and again in March, 1964. Both the previously studied site and the 'Beach Istana Area' consist of lalang fields located immediately behind a 15 yard wide strip of scrub vegetation which borders the sandy beach.

Relatively very few L. (L.) arenicola were recovered from trapped rats. Only one pool of 10 L. (L.) arenicola was studied for rickettsiae and this pool was negative. The remaining 54 specimens, all from R. r. jalorensis, and all except 2 chiggers from rats trapped at the Sultan's Beach House area, were mounted for identification. In Table 16 are summarized all collections made in the two study areas, including results of attempts to isolate R. tsutsugamushi from this material. The Beach Istana area clearly contains scrub typhus foci, whereas collections in the other area are too small to rule out its presence there. In the former area at least, studies to relate R. tsutsugamushi transmission to L. (L.) arenicola would have to be carried out so as to exclude even one L. (L.) akamushi from study material. This can be done with assurance only by collecting unengorged L. (L.) arenicola from the ground and feeding them on mice either singly or in pools, recovering the engorged chiggers post-feeding for identification.

Intensive black-plating was carried out in the 15 yard scrub vegetation strip behind the beach, where rats carrying L. (L.) arenicola were trapped. Only 14 L. (L.) arenicola specimens were recovered on black plates. It was felt that the number of this species of chigger present in the area was too low to justify further work at this site, so plans are now being made to transfer this work to Pangkor Island, where large numbers of this chigger have been recovered from rats.

Table 16

Summary of collections made at Morib for isolation of R. tsutsugamushi

Rat Species	No. rats trapped	Number of Rats carrying indicated chigger species					No. chigger pools inoculated			No. pools positive	
		L. (L.) akamushi	L. (L.) deliense	L. (L.) arenicola	A. (L.) indica	E. wichmanni	L. (L.) akamushi	L. (L.) deliense	L. (L.) arenicola	A. (L.) indica	E. wichmanni
Sultan's Beach House Area[1]											
R. argentiventer	1	0	0	0	1	0	-	-	-	1/6	-
R. jalorensis	5	1	1	4	3	0	-	-	1/0	-	-
R. exulans	2	0	0	0	0	0	-	-	-	-	-
Total no. of chiggers collected/inoculated	-	2	3	62	20	0	-	-	10	5	-
Beach Istana Area[2]											
R. argentiventer	9	9	0	0	1	1	8/8[3]	-	-	-[3]	-[3]
R. jalorensis	20	17	0	2	6	0	15/7[4]	-	-	5/0[4]	
R. exulans	1	1	0	0	0	0	1/1	-	-	-	-
Approx. no. of chiggers collected/inoculated	-	2700	0	2	740	15	2000			530	

1. Total Trap nights: 200
2. Total Trap nights: 500
3. 1 of 8 pools also contained some A. (L.) indica and E. wichmanni.
4. A single specimen of the identification sample from one A. (L.) indica pool and from one L. (L.) akamushi pool was L. (L.) arenicola. These specimens were the only individuals of this species recognized in this study area.

E. Transovarial Passage of R. tsutsugamushi in Vector Mites:-

Colonies of L. (L.) akamushi and L. (L.) deliense are maintained in the laboratory for the study of transovarial passage rate of scrub typhus rickettsiae. In Chart I experience with L. (L.) deliense is reviewed. It is presumed that the F_2 generation chiggers do not harbor R. tsutsugamushi. Chart II reviews experience with unengorged L. (L.) akamushi collected in January, 1965, at Subang, Selangor. No rickettsiae were recovered from these chiggers. The general area of collection at Subang had produced in the past, a few chiggers shown to have scrub typhus rickettsiae. Charts III, IV, V and VI, review experience with unengorged L. (L.) akamushi, collected in lalang a short distance off the road one half mile north of Kg. Jendram, Selangor (26th mile Kuala Lumpur - Sepang Road). Chiggers for establishment of colonies were sorted into 4 pools of 100 chiggers each. Each pool was fed on a single mouse, and R. tsutsugamushi was recovered from each mouse. The status of progeny of chiggers in these 4 pools is indicated in Charts III, IV, V, and VI.

F_1 larvae of one mated pari of adults derived from chigger pool (Chart VI) MF-1854 transmitted scrub typhus rickettsiae to normal mice upon which they fed. Each of twelve larvae were placed on single mice. Six larvae failed to attach and were lost. Six larvae attached, fed and were recovered. Each larva transmitted rickettsiae to its host mouse.

At this very early point in the work, no comment will be made on this finding. Naturally great effort will be expended to keep the six larvae alive to adulthood, in the hope that F_2 and subsequent generations of chiggers can be raised for study for scrub typhus rickettsiae. All other colonies outlined in Charts I through VI will be maintained. If further study proves them to be free of rickettsiae, they will be kept as clean chigger colonies for future work.

Chart I

L. (L.) deliense Chigger Colony
Origin: Subang, Selangor
Date: 24 April 1964

P: 100 larvae fed as pool (MF-1786)
(R. tsutsugamushi isolated from mouse on which pool of chiggers fed)

↓

5 adults, held as pool

↓

F_1: 51 larvae fed individually on single mice
(no rickettsiae recovered)

↓

8 live adults

↓

4 mating pairs of adults
[MF-1925/1933] [MF1936/1937] [1943/1959] [1958-1967]

↓ ↓ ↓ ↓

F_2 Larvae:

24	53	24	8
fed on mice individually	fed on one mouse as pool	fed on one mouse as pool	fed on one mouse as pool

Comment: This colony appears now to be free of R. tsutsugamushi. Succeeding generations will be tested for rickettsiae. (no rickettsiae recovered in mice used for feeding) Engorged F_2 larvae are now being raised in 4 separate pools.

Chart II

L. (L.) akamushi Chigger Colony

Origin: Subang, Selangor
Date: 7 January 1965

P: 100 larvae fed singly on individual mice (MF 1978-2077)
(R. tsutsugamushi not isolated from mice)

↓

16 (adults) held as pool → 10 eggs produced (30 Sep 65)

Comment: To be held as a pool, with F_1 larvae fed in pools. This colony is probably free of R. tsutsugamushi.

Chart III

L. (L.) akamushi Chigger Colony

Origin: Jendram Road (26th mile), Selangor
Date: 11 August 1964

P: 100 larvae fed as pool on one mouse (MF 1850)
(R. tsutsugamushi isolated from mouse)

↓

10 live adults

↓

4 mating pairs of adults

↓ ↓ ↓ ↓

F_1: 30 17 14 7

All larvae fed individually on single mice

Chiggers ↓ ↓ ↓ ↓
recovered: 15 13 11 5

(no rickettsiae isolated from mice fed upon)

Comment: It is possible that mating pairs have been derived from uninfected "P" larvae.

Chart IV

L. (L.) akamushi Chigger Colony

Origin: Jendram Road (26th mile), Selangor
Date: 11 August 1964

P: 100 larvae fed as pools on one mouse (MF-1851)
(R. tsutsugamushi isolated from mouse)

↓

13 live adults

↓

4 mating pairs of adults

↙ ↓ ↓ ↘

F_1: 4 16 10 22

All larvae fed individually on single mice

Chigger
recovered:

↓ ↓ ↓ ↓

4 15 10 20 (studies incomplete in 8)

(no rickettsiae isolated from mice fed upon)

Comment: It is possible that mating pairs have been derived from uninfected "P" larvae.

Chart V

L. (L.) akamushi Chigger Colony

Origin: Jendram Road (26th mile), Selangor
Date: 11 August 1964

P: 100 larvae fed as pools on one mouse (MF 1852)
(R. tsutsugamushi isolated from mouse)

↓

19 live adults

↓

6 mating pairs of adults

↙ ↓ ↓ ↓ ↓ ↘

F_1: 12 4 4 10 11 34

all larvae fed individually on single mice

Chiggers
recovered:

↓ ↓ ↓ ↓ ↓ ↓

11 4 4 10 9 34 (10 incomplete studies)

Comment: Status of F_1 larvae for R. tsutsugamushi is still unsettled, as of 30 Sep 65.

Chart VI

L. (L.) akamushi Chigger Colony

Origin: Jendram Road (26th mile), Selangor
Date: 11 August 1964

P: 100 larvae fed as pools on one mouse (MF-1854)
(R. tsutsugamushi isolated from mouse)

↓

26 live adults

↓

8 mating pairs of adults

↙ ↙ ↙ ↙ ↘ ↘ ↘ ↘

F_1: 8 24 20 40 17 22 12 19

All larvae fed individually on single mice

Chiggers recovered:

↓ ↓ ↓ ↓ ↓ ↓ ↓ ↓

8 24 12 36 13 14 6 15 (3 incomplete studies)

mice negative for rickettsiae — 6/6 chiggers transmitted R. tsutsugamushi to mice upon which they fed. — negative for rickettsial

F. Habitats of L. (L.) deliense in the presence and absence of L. (L.) akamushi

Previous workers in this Unit defined the habitats of L. (L.) deliense and L. (L.) akamushi at Subang, near Kuala Lumpur (Am. J. Hyg., 78:131-142, 1963): "Larval forms of L. (L.) akamushi and L. (L.) deliense have been shown to exist on the ground in distinctly different habitats. L. (L.) akamushi is found in grassland and L. (L.) deliense in relatively large numbers in forests." How generally applicable is this observation?

An opportunity arose to study this question in a joint field trip with the Thai Component, SEATO Medical Research Laboratory, Bangkok, Thailand, in August, 1965. It had been noted previously that relatively large numbers of both species of chiggers had been collected from rodents trapped in only one area of Thailand, Kao Yai National Park, about 100 miles north-east of Bangkok, at an elevation of about 2000 feet. This area topographically is very similar to Subang, Selangor, in that tracts of lalang grass and secondary forest are interspersed.

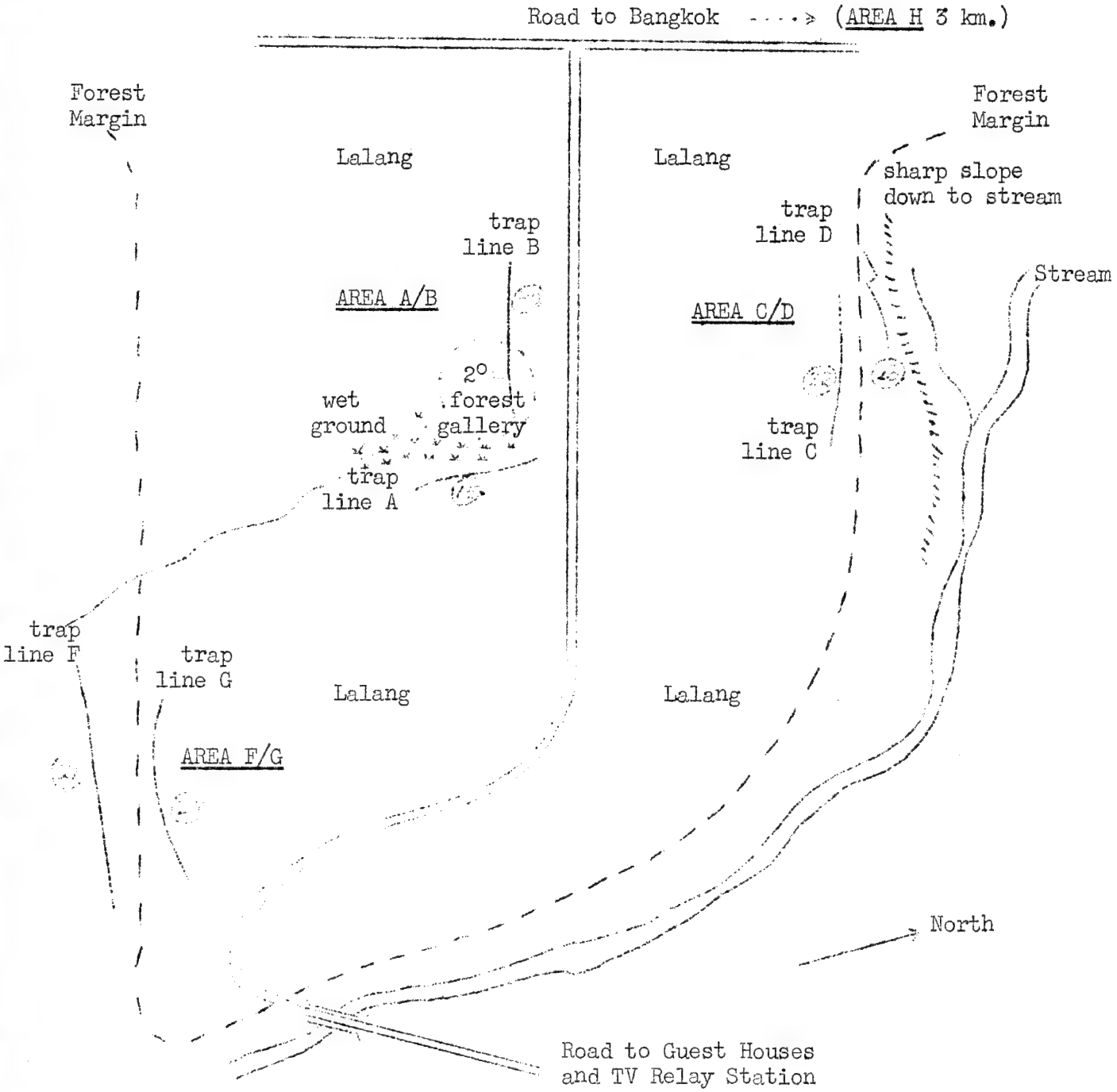
Collections of chiggers from the ground were made between 17 and 20 August. In Sketch I, the study areas are shown. In Table 17 the results are summarized. The distribution of both chiggers on the ground was exactly the same as at Subang.

While in Thailand, an opportunity also arose to study, by black-plating, an area of lalang and secondary forest, known to contain a scrub typhus focus. Here only L. (L.) deliense was found, in both lalang and secondary forest. This finding has made us wonder how generally applicable is the following: when both chiggers occur together, they are mutually exclusive as to habitat, L. (L.) akamushi confined to lalang and L. (L.) deliense confined to surrounding forest; when L. (L.) deliense only is found in an area, its distribution includes the habitat occupied in other areas exclusively by L. (L.) akamushi. Studies are planned for the coming year to examine this question in Malaya.

SKETCH I

Kao Yai National Park

17-20 August 65



note: Blackplating was done along trap lines

Circled figures indicate number of traps on each trap line.

Table 17

Summary of Black Plate Collections of
L. (L.) akamushi and L. (L.) deliense
at Kao Yai N.P. Thailand, Aug 65

Study Area - date		Lalang floor		Forest floor	
		akamushi	deliense	akamushi	deliense
A/B	17 Aug	2	1	0	19
	18 Aug	126	0	0	45
	20 Aug	69	0	-	-
C/D	17 Aug	17	0	0	0
	18 Aug	4	2	0	4
F/G	19 Aug	19	0	0	76
T O T A L S		237	3	0	144

G. Search for Encephalitozoon cuniculi in the Normal Mouse Colony

Encephalitozoon cuniculi (E.c.) is a protozoon-like agent which has been found in normal mice, guinea pigs, rabbits and other mammals. Japanese workers described what is called interference between Encephalitozoon cuniculi and R. tsutsugamushi (Yokohama Med. J., 9:412-420, 1959), manifest by loss of rickettsiae when inocula containing both agents are serially passed in mice. In view of the type of critical isolation studies conducted in our scrub typhus investigations, it was considered essential that the status of the normal mouse colony with respect to E.c. be determined.

Accordingly, a sequence of five serial passages of mouse tissue suspensions at one month intervals was initiated. From 100 mice, divided into ten pools of ten mice each, brain, liver, and spleen were harvested to prepare ten pooled tissue suspensions. Each tissue suspension was inoculated intraperitoneally into ten mice. On the 28th day post-inoculation, the mice were sacrificed, tissue suspensions prepared as before from each group of ten mice, and passed to ten mice each. By this passage technique, it is possible to amplify E.c. in mice to produce ascites and hepatosplenomegaly between the 21st and 28th days post-inoculation. At each passage level, one half of the brain of each mouse was fixed in Zenker's acetic acid solution and stored for possible future histologic study.

A total of 600 adult mice were studied in this way. None of the mice showed signs of E.c. infection. In view of the failure to uncover any clinical signs of infection, it was decided unnecessary to study histologically the brain tissue which had been fixed and stored. Further efforts to isolate this agent from normal mice will not be made at this time. We shall be alert for the possible introduction of this agent into the mouse colony by inoculation of field-collected material.

Investigations of Tick Typhus

In May, 1965, collection of ticks was attempted in Krau Game Reserve, Central Pahang. The area had been entered by Commonwealth Forces in June, 1964, for jungle training. One man developed fever 7 days after entering the training area and removed a tick 5 days before onset of fever. Ticks were very numerous in one zone of the training area. The man's illness was proven serologically to be Tick Typhus by CF test employing Spotted Fever rickettsiae antigen prepared at WRAIR. Ninety-six adult ticks were collected in 4 days of tick-dragging. These were sorted and inoculated in pools as follows: 6 pools of Haemophysolis semermis, 2 pools of Haemophysolis papuana, 1 pool of Haemophysolis cornigera, and 1 pool of Dermacentar sp.

A proportion of ticks in each pool were retained uninoculated for the reference collection.

The ten pools of ticks were inoculated into 3 guinea pigs each. Disease in guinea pigs typical of spotted fever rickettsial infection was not observed. Each isolation line was passaged once at 14 days. Serum from non-sacrificed animals did not contain spotted fever antibody. It is of interest that tick typhus rickettsiae were recovered from ticks Haemophysolis semermis and Haemophysolis papuana collected in Gombak Forest Reserve, Selangor in 1963. These two species comprised most of the collections made in Pahang.

Similar epidemiologic investigation of other cases of tick typhus will be undertaken, as cases are recognized.

Investigations of Malaria

A. Sporozoite Immunity

1. Purpose:

Our main objective in this study is to investigate the feasibility of inducing in monkeys immunity to malaria by prior inoculation of killed sporozoite.

2. Materials and Method:

Rhesus monkeys from malaria free areas of India are employed. Thick smears are employed to ensure negativity. Pre-inoculation blood is collected for fluorescent antibody tests. Mosquito glands containing sporozoites are dissected and stored in serum saline solution in -65°C when sufficient numbers are obtained, they are inactivated using the rapid thawing-freezing methods. 1% formalin is next added to obtain 0.1% solution. Equal volumes of Freunds complete adjuvant is added to the inoculum which is administered subcutaneously. Monkeys A1, A2, F1, G1, G2 and H1, received inoculum not containing Freund's adjuvant. In the last four monkeys it was omitted because of the suspicion that it might be related to the development of chronic suppurative lesions occurring both at sites of inoculation as well as in the viscera. These lesions were not encountered in monkeys not receiving Freunds adjuvant (see Table 18) control monkeys receiving negative glands are treated in parallel fashion. The monkeys are next challenged by two infected mosquitoes. Examination of blood smears are carried out daily.

3. Results:

Clinical immunity is assessed by determining the effect if any on the duration of the pre-patent period and the degree of the ensuing parasitemia.

Table 18

Summary of Treatment

Monkey No.	Day of Treatment/No Glands						Tot No glands	Day Challenge	No Mosq /Challenge	Equal Vol Adj-uvant added	Pre-patent Pd. days +
	Day 1	Day 8	Day 15	Day 21	Day 29	Day 57					
A1	164+	190+	192+	-	-	-	549	22	3	No	10
A2(c)	155-	170-	180-	-	-	-	505	22	2	No	10
B1	333+	-	334+	-	335+	-	1002	43	2	Yes	10
B2(c)	333-		332-		300-		965	85*	2	Yes	8
C1	334+				335+	335+	1004	85	2	Yes	22
C2(c)	333-				300-	334-	967	85	2	Yes	10
D1	500+				501+	505+	1506	169*	2	Yes	13
E1	333+				335+	217+	885	192*	2	Yes	12
F1	505+	205+	198+	209+	207+	-	1324	45	2	No	12
G1	205+	200+	200+	200+	194+	-	999	46	2	No	14
G2(c)	199-	200-	206-	220-	205-	-	1030	39	2	No	10
H1	210+	210+	200+	210+	240+	-	1070				

(c) Negative control

* B2 1st challenge on day 43 considered unsatisfactory
D1 1st challenge on day 81 considered satisfactory
E1 1st challenge on day 91 considered satisfactory

\+ Prepatent Period reckoned from day of challenge to day when parasitemia is first noted.

a. Pre-patent period:

This is reckoned from the day of challenge until the day when parasitemia is first noted. Taking 13 normal monkeys, challenging them in the usual manner, the average pre-patent period for the group was 10.5 days, with the range of between 9 and 14 days (see Table 19). The pre-patent period in the monkeys receiving negative glands was not dissimilar. However in the experimental group an overall lengthening of the pre-patent

period is evident. It must be pointed out that monkeys D and E1 (see Table 18) did not come down with infection despite what was considered adequate standard challenges initially. The resultant delay was not considered in the tabulation of the pre-patent period as shown in Table 19. When these monkeys were rechallenged in the usual manner at a date considerably removed from the last inoculation of inactivated sporozoites, these monkeys came down with infection. The pre-patent periods were 13 and 12 days respectively. Also of interest is the observation that the average pre-patent period of the last five consecutive experimental

Table 19

Pre-patent Period in 3 Groups of Monkeys

Monkey Group	No.	Ave. Pre-patent period days	Range days
Normal	13	10.5	9 - 14
Neg Cont	4	10.0	9 - 11
Exp	7	13.7	10 - 22

monkeys was 14.6 days with the range of between 12 and 22 days. Monkey A1 the first in the experimental group was considered to have received insufficient number of glands (549) and given at too frequent an interval (1 week) of 3 inoculations to provoke antibody production. Despite the fact that B1, the second experimental monkey, received adequate number of inactivated glands (965), the spacing of 3 inoculations at two week intervals was obviously not optimal for immunity to develop. Subsequent inoculations were given at monthly intervals (C1, D, E1) with total glands ranging from 885 to 1506. Monkeys F G1, G2 and H1 received about the same number of glands but given at weekly intervals spread out into 5 inoculums.

(b) Degree of parasitemia

To study the degree of parasitemia we have arbitrarily elected to consider a 50 day period of observation commencing from the day of challenge. In nearly all the monkeys both control and experimental the parasitemia is reduced to a low level by this 50-day period. No clear cut pattern emerges in either group beyong this period. The daily counts may be classified according severity. Thus 1+ infection involves between 0 - 10,000 parasitized cells per 1mm3 2+ between 10,000 to 50,000, 3+ between 50,000 to 100,000 and 4+ greater than 100,000. Table 20 shows the break-down of the daily counts in this manner during the 50-day observation period.

Table 20

Break-down of daily counts according to severity

Monkey group	No/Gr.	Ave No. days with pos. counts/50 day pd.	Distribution of daily counts according to intensity			
			1+	2+	3+	4+
Normal Cont.	13	40.4	12.6	11.1	7.1	9.6
Neg Cont	4	41.0	15.3	9.0	7.3	9.5
Experim. A1 - G1	7	35.9	15.0	11.7	6.0	3.0
Experim C1 - G1	5	33.8	15.2	14.2	4.2	0.2

Note that the average number of days with positive counts during the 50-day period is comparable in both the control groups. This is not so in the experimental group being slightly reduced (35.1 days). The reduction is even greater when the last 5 consecutive test animals are observed. Accounting for the reduction are (1) the slight but definite prolongation of the pre-patent period and (2) absence of parasitemia during part of the course in E1 during the period of observation. As for the severity of the infection in the control animals is concerned, there were on the average, almost identical numbers of moderately severe (3+) and severe (4+) parasitemias. However in the experimental group the course of the ensuing infection was milder. This is even more impressive in the last five consecutive test monkeys. Only one count of 100,000 parasites /mm3 or greater was recorded amongst them. Moderately severe counts were likewise reduced.

Effect of Freunds complete adjuvant

Freunds complete adjuvant consists of (a) mineral oil (b) lanolin (c) old tuberculin material. It was hoped that addition of this material would delay absorption of the inoculum from the subcutaneous sites thereby producing a prolonged stimulatory effect on antibody production. Indeed the absorption of the inoculum was delayed in all those monkeys receiving it. Approximately 10 days following injection a small pustular lesion is observed. This gradually enlarges and suppurative material begins to drain. Similar lesions may develop distal to inoculation sites. Numerous attempts at culturing these lesions yielded no pathogenic organisms. These lesions may last several weeks (12-16 weeks) before they heal gradually. Monkeys C1, D1 and E1 despite the suppurative lesions, came down with only very mild malarial infection after challenge. Several weeks after the 50 day observation period C1 gradually deteriorated despite the fact that its malaria remained subclinical.

It failed to gain weight. Its fur was noted to straighten lacking the luster of the normal monkey fur. There is in addition patchy areas of hair loss (alopecia). Circumoral and periorbital pallor begin to show soon afterwards. Conjunctival injection is also apparent. The animal becomes very timid. At post mortem - on C1 numerous abscesses were noted in lungs, liver, lymph nodes, spleen, kidneys. Culture of material for AFB, Ps. pseudomallae and other bacteria were negative. Histologically these involved areas showed casseous necrosis with giant cells like that seen in tuberculosis. Strain for amyloid was also negative. As of the report, monkey E1 is similary deteriorating despite partial healing of its suppurative lesions. A tuberculin skin test was negative in E1. Monkey E1 will be closely observed. In the meantime Freunds adjuvant has been omitted in monkeys F1, G1, G2, H1. No suppurative lesions occurred in these.

Demonstration of Antibodies

(a) X-globulin rise - using paper electrophoresis it is possible to demonstrate a rise in the X-globulin fraction of the serum proteins in monekys treated with inactivated glands. A small rise is noted in the monkeys receiving negative glands as well. (see Table 21).

Table 21

Serum Protein Distribution

Monkey Group	No/Gr.	Albumin	Alpha 1	Alpha 2	Beta	Gamma
Normal	11	56	6	8	15.2	15.1
Neg Control	3	55.6	6.8	7.5	12.5	17.3
Experimental	6	46.1	5.9	6.6	16.1	24.4

A fairly impressive rise is noted in the 6 experimental monkeys. This rise is mostly at the expense of the albumin fraction. A greater rise was observed in those monkeys which received inocula containing Freunds adjuvant. Despite a technical difficulty at clear separation into the component fractions a definite elevation of the X-globulin fraction is observed.

(b) Agglutination titer

Clumping of fresh sporozoites in the presence of specific serum can be demonstrated, in this case by using serum from monkeys treated with inactivated sporozoites. The pre-treatment sera serves as a control. Sera obtained from monkeys after the last inoculation of inactivated sporozoite and just before mosquito challenge are considered as positive. Positive sera from C1, E1, and F1, all gave titers up to 1:124. Addition of a drop of diluted methylene blue provides greater clarity of the clamping phenomenon.

(c) Cross-protection

Serum from E1 which was found to have agglutination titer of 1:124 was given subcutaneously to fresh monkey, E3 (see Table 22). Serum obtained from E3 was given in like manner to another fresh monkey E4. The dose is as indicated.

Table 22

Monkey	Wt	Source Serum	Amt/route	Day Chall.	Method Chall.	Prepat pd.	Course
E-3	2.7 Kg	E1	3-7cc/Kg SQ	2	2 mosq.	9	mild
E-4	1.8 Kg	E3	3-6cc/Kg SQ	2	2 mosq.	10	mild

Each of the monkeys was exposed to two infected mosquito challenges on day 2. The pre-patent periods for E3 and E4 were 9 and 10 days respectively. The ensuing course of infection was not different perhaps a little milder in monkey E4 that received normal serum. Cross circulation experiment using monkey G1 and G3 in the hope of exchanging blood after their blood were found to match both in the major type as well as to some of the minor factors, was tried. The experiment had to be abandoned because of the small caliber of the femoral vessels for cannulation.

(d) Fluorescent Antibody Technique

Using the indirect method, fresh sporozoites as an antigen, and serum from treated monkeys, and goat anti-monkey globulin conjugated to fluorescene, we are able to demonstrate fluorescence of the sporozoites. The fluorescence is not very bright. Only the outer wall of the sporozoite fluorescence leaving the body bare. Further, there is considerable reduction in the number of fluorescing sporozoites. Attempt is being made to rectify this situation by altering the method of preparing, and fixing the antigen.

Of some concern is the presence of glandular fluorescence. This is very striking. It will be recalled that in the preparation of the inoculum it has not been possible to separate the glands from the sporozoites. This glandular material may act as an antigen and therefore account for the subsequent fluorescence when fresh sporozoites with the salivary glands are used as an antigen again. Of greater concern is the fact that this glandular fluorescence may also be observed when serum from normal control monkeys are used. No obvious reason to account for this phenomenon is forthcoming. It is possible that these monkeys have been exposed to mosquito glandular substance during previous mosquito bites.

B. Malaria and Genetic Factors

This study was initiated in 1964. The purpose was to determine the influence if any, of the various genetic factors, namely glucose-6-phosphate dehydrogenase, transferrins, hemoglobin type and haptoglobin type on malaria infection. Our study was initially confined to the aborigines because of the presumed higher incidence of malaria among them. This was not found to be the case. During the year two surveys were carried out in aborigine settlements namely Suak Padi, Perak and Ulu Langat, Selangor. The respective malaria incidence were 0% (0/167 examined) and 16.3% (7/43 examined). Thus far only 52 aborigines with malaria have been assessed for genetic factors mentioned above. The number is considered too small to warrant conclusion. Efforts to locate other areas, not necessarily aborigine settlements, are being made. Two one-day surveys in the State of Selangor between April and May 1965 again showed malaria incidence of less than 6% and therefore the genetic studies were not carried out. Malaria incidence of between 30-50% have been found in few villages in Upper Perak. This information was obtained through the courtesy of the Malaria Pre-eradication Program of the Ministry of Health, Malaysia. Study of these areas are more apt to yield information relative to genetic factors and malaria and hence a field trip to Lenggong, Upper Perak is being considered.

C. Malaria Survey

A peninsula wide malaria survey is currently being undertaken by the Malaria Pre-eradication Program, Ministry of Health, Malaysia in conjunction with the World Health Organization. This survey is expected to be completed in March 1966. Thus far the states of Selangor, Negri Sembilan, Malacca, Perak, Kelantan and Trengganu have been surveyed (Third W.H.O. Quarterly Report 1965 - Malaysia 20)

The USAMRU participation in the survey has been limited to the reading of approximately 1000 smears per month. As a result of this effort it has been possible to locate specific areas of malaria incidence in Malaya. It was thus shown that the villages of Lawin, Malau, Belum Bharu and Sawa in Upper Perak State had a malaria incidence of between 30 and 50%. Plasmodium Falciparum represented the predominant species.

Studies of the Aborigines

Clinical Studies

During the year a research ward of twenty beds was established in an existing ward building at the Aboriginal Hospital at Gombak, Selangor, on the fringe of the jungle thirteen miles east of Kuala Lumpur. The ward was staffed to provide twenty-four hour medical and nursing care for in-patients: one physician (British), five nurses (three British, one Australian, and one American) and eight aboriginal ward attendants. Laboratory, x-ray and secretarial space was created in a second building and was staffed by two technicians and one secretary. A clinical pathology laboratory was established at the IMR in space occupied in previous years by other grant activities and was staffed with one physician (clinical pathologist from the Philippines) and three technicians. The research objective of this program was the etiologic study of PUO's occurring in aboriginal people. It was hoped also that anemias found in study patients might be investigated etiologically.

During the first nine months of ward operation, 80 patients were admitted. These patients were selected from patients brought to the hospital by helicopter and road transport from jungle settlements. Because only the more severe illnesses are evacuated to Gombak from most of the aboriginal settlements, cases suitable for PUO study were infrequently encountered. Four PUO's were admitted, two classical dengue fevers, and two others, also of probable viral etiology. The remainder of the patients admitted to the ward had a wide variety of general medical problems. Among these were encountered four macrocytic anemias related to malnutrition, one hemolytic anemia of unknown cause, and six anemias related to various infectious disease states. It was not possible, because of limitations of professional staff, to use available helicopter service to seek out in the jungle patients suitable for study and transport them to the hospital for work-up.

It is planned to confine future clinical studies of the aborigines at Gombak Medical Center, and in the jungle, to investigations of diarrheas of bacterial origin. Included will be studies of whole communities of jungle-dwelling aborigines.

Genetic Studies

Because the Unit was engaged in a study of the relationship of certain genetically determined traits to susceptibility of Malaysian to malaria, the aborigine patients admitted to the ward at Gombak were also studied to determine their glucose-6-phosphate dehydrogenase activity and hemoglobin type. Ten of 68 patients studied were found to be deficient in glucose-6-phosphate dehydrogenase activity. Five of 65 patients studied were found to have only hemoglobin type E, and an additional 15 patients had both types A and E. No clinical disease could be ascribed to either the enzyme

or hemoglobin abnormalities found in these patients.

In the course of malaria work, a number of aborigines were examined for various genetically-determined traits. In Table 23, findings to date in studies of hemoglobin are summarized. Patients seen at Gombak Medical Center are omitted because of their diverse geographic origin.

Table 23

Hemoglobin Types of Malayan Aborigines

Aborigines Group	Location of study	No. Individuals studied	Hemoglobin types (% of total) A	A-E	E	F	A
Aboriginal Malays	Carey Is. Selangor	137	137				
	Ulu Langat Selangor	42	42				
Senoi	Penderas, Central Pahang	182	151	29(16%)		2	
	Suak Padi, south lowland Perak	252	112	116(46%)	23(9%)		1
Total individuals studied		571					

The difference in incidence of homozygous and heterozygous E in the various groups of aborigines is striking. Further geographic study will be undertaken.

The findings to date in studies of glucose-6-phosphate dehydrogenase activity are summarized in Table 24.

Table 24

Glucose-6-Phosphate Dehydrogenase Deficiency in Malayan Aborigines

Aborigines Group	Location of study	No. Individuals studied	G-6-PD Activity Normal	Deficient
Aboriginal Malays	Carey Is. Selangor	59	56	3
Senoi	Penderas, Central Pahang	103	67	36
	Suak Padi, south lowland Perak	121	90	31
Total individuals studied		283	213	70

Extension of this work is planned in association with serologic studies of aborigines for scrub typhus antibody. Liaison is to be established with Dr. Lie-Injo Luan Eng, now of Hooper Foundation, University of California who undertook the initial screening surveys several years ago.

Additional studies of Malaya and Tamil Indians relative to hemoglobin type, G-6-PD deficiency, haptoglobin type were undertaken during the year. When representative samples of these populations have been studied, results will be presented.

Additional Studies

Cholera Survey

A brief survey of a cholera outbreak in Trengganu which occurred in May and extended up to September 1964 was undertaken by the USAMRU and IMR staff between July 28 and August 8 1964. Our main objective was to seek out pre-disposing factors that might have been responsible for the outbreak to occur in some villages but not in others. A questionaire was prepared embodying 92 items designed to bring out these various factors. One hundred and ninety seven separate household members were interviewed from eight different kampongs. A total of 170 rectal swabs from five kampongs were obtained. All were found to be negative for vibrio cholera. Forty duck and chicken and 1 monkey (M. Irus) rectal swabs were likewise obtained from these various kampong. One duck rectal swab yielded a characteristic colonial morphology of V. cholera which when stained showed curved rods. It did not however agglutinate with polyvalent cholera antiserum.

Literacy, nutritional status, type and source of drinking water, methods of disposal of human and vegetable waste employed, presence of domestic animals and a prior drought did not materially affect the cholera incidence or distribution in the kampongs. Travel habits of the inhabitants of the villages were uniform and therefore of little consequence in the patchy distribution of the cases.

In kampongs where half or more of its inhabitants who did not boil or cover drinking water a greater accumulation of cases was noted. An occupational hazard was suggested in that padi planters seemed vulnerable. However this did not prove to be so when examined statistically. Adults over twenty years of age were found to be particularly susceptible and children under 9 resistant, the observation being statistically significant. An overall analysis of the entire outbreak in terms of its susceptibility and resistance in individuals of different age groups seems worthwhile.

Investigations: Migratory Animals Pathological Survey

Netting and banding of birds continued throughout the year. Approximately 18,000 birds were banded by employees of the M.A.P.S. project, and a further 2,500 by amateurs and associated groups in other parts of Malaysia, representing an increase of nearly three-fold over the previous year. Bloodsmears, ectoparasites, and the carcasses of accidental bird casualties were routinely collected. A list of chiggers identified by U.S.A.M.R.U. staff is attached (Table 25), together with lists of Mallophaga (Table 26).

Two new bird species for the Malayan list were netted during the year. Both are Palaearctic migrants: the Red-breasted Flycatcher (Muscicapa parva), and the Red-rumped Swallow (Hirundo daurica).

The great increase in numbers of birds netted was due largely to successful trapping in two habitats previously uninvestigated: (a) Swallows roosting on power lines in urban areas, and (b) nestling Night Herons in a heronry in the mangrove in northwest Perak.

Approximately 12,000 swallows were netted during the year, principally in the neighbouring towns of Bentong and Raub, Pahang. Retraps of our own birds show that both resident swallows (Hirundo tahitica) and the migratory species (Hirundo rustica), the latter constituting 85% of all swallows handled, move freely between these two towns. Birds ringed in one town on a given night have been recaptured in the other town on the following night. So far, no long distance recoveries have been reported.

In the mangrove off Kuala Gula, Perak, at 4° 55'N, 100° 35'E, 1186 nestling Night Herons (Nycticorax nycticorax) were banded during the year. Of these 13 recoveries have so far been reported, all by members of the public - a percentage recovery considerably above expectation even in countries like the U.K. or U.S.A. The locations of all recoveries (Table 27) range from about 80 miles south to about 100 miles north of the heronry. The distribution of recoveries indicates that, on post-juvenile dispersion, the fledgling Night Herons effectively cover the northwest coastal plain of Malaya from Lower Perak District to maritime parts of Kedah. Since in other parts of its range the Night Heron is known to be a natural reservoir of Japanese encephalitis, the discovery of this pattern of distribution could very well be of epidemiological importance. One nestling brought back to Selangor and raised until it flew was recovered in Negri Sembilan, some 30 miles southward.

Table 25

Bird Ringing Project (Malaysia)
Chiggers identified by USAMRU staff

Host	Ring No.	Total chiggers	Chiggers identified# (1)	(2)	(3)	(4)	(5)
Ixobrychus sinensis*	080.00024	12				12	
Rallus striatus	060.00207		1			19	
Porzana fusca	050.00304		2				
Centropus bengalensis	080.00001	4				4	
	080.00002	114				20	
	080.00021	502				50	
	080.00039	45	3			42	
	060.00203	96				20	
Pitta sordida	030.00120	4	4				
	030.00455	2					2
Pellorneum capistratum	030.00416	4	4				
Trichastoma malaccensis	020.00002	2	2				
	020.00019	1	(damaged)				
Trichastoma abbotti	030.00119	6	6				
Stachyris poliocephala	030.00002	4	4				
Stachyris nigriceps	030.00421	3	3				
	BA 16634	5	4				
Stachyris leucotis	040.00296	11	5	4			
	040.00297	6	1	5			
	030.00405	22	8	6	8		
Stachyris maculata	030.00122	1	1				
	030.00123	2	2				
Stachyris nigricollis	-	4	2			2	
Alcippe castaneiceps		2	1	1			
Locustella lanceolata*	020.00380	9				9	
Copsychus malabaricus	030.00424	2	2				

#(1) Leptotrombidium (L.) deliensis (Walch).
(2) Leptotrombidium (L.) bodensis
(3) Leptotrombidium (L.) keukenshrijveri
(4) Blankaartia acuscutellaris
(5) Toritrombicula

(*) Migrant hosts

Table 26

Mallophaga from Malayan avian hosts

Alcedoecus sp.	
DS 09262	Halcyon pileata*
Alcedoffula sp.	
N 24127	Ceyx rufidorsus
Bruelia sp.	
N 24223	Lonchura atricapilla
Myrsidea spp.	
020-00014	Serilophus lunatus
BA 29761	Acrocephalus arundinaceus*
N 24270	Rhipidura javanica
N 24265	Lonchura atricapilla
BA 29758	Ploceus philippinus
N 24191	Rhipidura javanica
020-00010	Copsychus malabaricus
BA 29572	Pychonotus zeylanicus
N 24209	Rhipidura javanica
020-00025	Muscicapa hyperythra
010-00015	Hypothymis azurea
010-00029	Rhipidura albicollis
Myrsidea rustica (Giebel 1874)	
N 24413	Hirundo rustica*
N 24439	Hirundo rustica*
N 24418	Hirundo rustica*
Philopterus	
BA 29712	Acrocephalus arundinaceus*

(*) Migrant hosts

Table 27

Recoveries of Night Herons banded at the heronry near Kuala Gula, Perak

Date ringed (1964)	Date recovered (1964)	Locality
8 October	13 November (1965)	Butterworth, Province Wellesley
4 November	January	Yen Besar, Kedah
18 December	7 February	Telok Anson, Perak
9 October	10 February	Parit, Perak
18 December	24 February	Padang Manora, Penang
18 December	25 February	Jarak Atas, Province Wellesley
18 December	28 February	20 miles from Parit, Perak
18 December	3 March	Bruas, Perak
4 November	31 March	Selinsing, Bagan Serai, Perak
17 December	14 April	Sungei Limau, Yen, Kedah
4 November	22 April	Kg. Sanglang, Kedah
18 December	4 May	Kg. Pinang Tinggal, S. Patani, Kedah
8 October	July	Kuala Kurau, Perak

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2. Gentry, J.W. Miniature radio-tracking studies of the hosts of vector mites in Malaysia. J. Med. Ent., 2:153-156, 1965.